सत्यमेव जयते

INDIAN AGRICULTURAL
RESEARCH INSTITUTE, NEW DELHI

36739

I.A.R.I.6.
GIP NLK—H 3 I.A.R.I.—10.5.55—15,000

THE BOTANICAL REVIEW

VOL. XV JANUARY, 1949 No. 1

THE MALE GAMETOPHYTE OF ANGIOSPERMS*

P. MAHESHWARI
Dacca University, India

Introduction 1
The microspore tetrad 5
First division of the microspore 7
The generative cell 11
Division of the generative cell 15
The male gametes 21
The vegetative nucleus 28
The pollen tube 30
  Germination of pollen 30
  Course of the pollen tube 32
  Entry of pollen tube into embryo sac 36
  Branching, persistence and other abnormal behavior of pollen tube .. 37
  Number of pollen tubes entering an embryo sac 40
  Rate of growth of the pollen tube 42
Participation of male cytoplasm in syngamy 47
Embryo sac-like pollen grains 51
Systematic value and homologies of the male gametophyte 53
Acknowledgments 58
Literature cited 59

INTRODUCTION

Although Amici, Robert Brown, Von Mohl, Hofmeister and several other botanists, working in the first half of the nineteenth century, saw germinating pollen grains and streaming cytoplasm inside pollen tubes, they had little or no knowledge of their nuclear contents and believed that fertilization occurs by diffusion of the sap ("fovilla") from the tube into the embryo sac. Schleiden erroneously thought that the pollen tube itself gives rise to the embryo sac.

Reichenbach (1852) and Hartig (1866) are generally considered
to have be nuclei inside the pollen grains
of *Tradesc* *thera*, *Lilium* and some other

---

* This art the morphological and cytological
features asso nd organization of the male gameto-
phyte. The aspects of pollen germination and
pollen tube g only incidentally.

36739
IARI

genera[1]. The latter used the simple technique of mounting nearly mature pollen in a drop of carmine-glycerine and letting the preparation stand 12 to 24 hours.

Hartig's work remained almost unnoticed, however, until Strasburger (1877) called attention to it in a memoir entitled "Über Befruchtung und Zellteilung". He extended Hartig's observations to include several other angiosperms and established the occurrence of the binucleate condition as a general phenomenon. He further showed that one of these nuclei originally lies in a small cell cut off at the periphery of the pollen grain but becomes free in later stages by a dissolution of the partition wall. Strasburger also germinated various pollen grains in artificial media (Van Tieghem, in 1869, and some others had accomplished this before him but without paying much attention to the nuclei inside the tube) and noted that the nucleus of the larger cell is always in the distal part of the pollen tube. He made the mistake, however, of thinking this to be the generative and the nucleus of the smaller cell to be the vegetative nucleus.

Strasburger's pupil, Elfving (1879), made similar observations on a number of other plants. When he could not germinate the pollen grains in artificial media, he made preparations of the pollen tubes from dissected styles. He confirmed Strasburger's account and said [free English translation]: "The pollen grain, originally provided with a spherical nucleus, becomes divided into two cells, of which the smaller is almost always found in one corner. . . . No trace of a cellulose membrane could be detected between the two sister cells. . . . Even the separating plasma-layer disappears in later stages, and in the mature pollen grain the two nuclei lie free beside one another". Concerning the pollen tubes he said: "I have tried to follow the nuclei until the moment of fertilization. In many cases one of them divides again, so that there are three nuclei. It always seemed to me to be the posterior nucleus which divides, and in *Orchis maculata* I can state positively that this is the case".

Elfving believed the nucleus of the larger cell to be of greater importance than that of the smaller or "vegetative cell" ("Die grosse Zelle des Korns und ihr Kern scheinen vom grösserer Bedeutung für die Befruchtung als die vegetative zu sein"). He advanced the following arguments in support of this view: (*a*) it is the larger cell which forms the pollen tube; (*b*) in most cases it is the nucleus of this cell which goes in front; and (*c*) in some instances the smaller cell remains in its original position without entering the tube at all[2].

---

[1] See, however, Wulff (1933), according to whom Meyen (1839) was the first to figure correctly the structure of the pollen grain of *Fritillaria imperialis*, and later Nägeli (1844), Wimmel (1850), Hofmeister (1861) and Tschistiakoff (1876) did the same in some other plants.

Elfving, like Strasburger, therefore, made the mistake of supposing that the smaller cell in the pollen grain was the vegetative. He added that by further division this could give rise to a two- to three-celled tissue or to "free-swimming vegetative cells" ("freischwimmende vegetativen Zellen").

Regarding the pollen grains of the Cyperaceae, he supposed that the three smaller nuclei at the narrower end of the pollen grain, now known to be the nuclei of the nonfunctioning microspores, are vegetative, and that the two larger nuclei at the broader end, now known to be the vegetative and generative, are derived by a division of the central nucleus. One of these was observed to divide again to form two daughter nuclei, now known to be the male gametes.

In spite of these misinterpretations it must be conceded that with the crude technique of those days Elfving gave some remarkably accurate drawings of the contents of the pollen grains and pollen tubes of a number of plants. A colossal and most unfortunate mistake made by him, however, was that he thought that all the nuclei dissolved sooner or later and, in any case, before fertilization took place ("Die Kerne werden früher oder später, doch vor der Befruchtung aufgelöst").

In a well known paper entitled "Neue Untersuchungen über den Befruchtungsvorgang bei den Phanerogamen", Strasburger (1884) corrected most of these mistakes and gave the first clear account of the history and development of the male gametes which he designated as "generative" cells or nuclei. He stated that due to its similarity, in position and origin, with the prothallial cells of gymnosperms, the small lenticular cell in the angiosperm pollen grain was formerly misinterpreted by him as being the "vegetative". His more recent work showed that the reverse was the correct interpretation, *i.e.*, the smaller cell was the generative and the larger the vegetative. Further, in the previous work in his laboratory, which had been done with whole mounts stained with iodine green, the nuclei in the pollen grains of several dicotyledons were often quite indistinguishable and had therefore been supposed to have degenerated. Improved methods used after 1877, such as staining with picrocarmine and cutting the larger pollen grains into thin sections, had shown that such was not the case.

---

[2] In this respect Elfving's observations were incorrect; or, possibly he was examining some abnormal pollen grains.

In conclusion he said that the first division of the microspore ("progame Pollenzelle") gives rise to two cells, the smaller being the generative and the larger the vegetative. The nucleus of the latter was frequently observed to take peculiar forms, sometimes becoming extremely thin and filamentous. The generative cell loosens itself from the wall of the pollen grain and often becomes spindle-shaped. It can divide either before germination of the pollen grain or afterwards. The vegetative nucleus, on the other hand, remains undivided. Previously recorded instances of its division, as reported by Elfving in the Cyperaceae, were to be interpreted differently. Here the three small cells at the narrower end of the pollen grain are really non-functioning members of the tetrad which are soon absorbed. The nucleus of the functioning cell alone divides to form the vegetative and the generative cells, and the latter divides again to form the two male gametes. The Juncaceae were found to be similar except that here all four members of the tetrad function and develop up to the three-nucleate stage.

In other plants, where the pollen grains are shed at the two-celled stage, division of the generative cell was found to occur in the pollen tube, so that in every case the tube eventually has three nuclei—one vegetative nucleus and two generative (or male) nuclei. These do not dissolve, as was previously supposed, but are carried by the pollen tube into the embryo sac. The most important event, which was found for the first time, was the entry of one of the male nuclei into the egg cell and its fusion with the nucleus of the latter.

Strasburger thus provided the first demonstration of syngamy in angiosperms. He did not know the fate of the second male gamete, however, and believed that it degenerated. Nawaschin (1898) and Guignard (1899) showed a few years later that it enters into a "triple fusion" with the two polar nuclei, resulting in the formation of the primary endosperm nucleus.

At the beginning of the 20th century a fairly clear picture had, therefore, been obtained of the structure and development of the male gametophyte. Coulter and Chamberlain (1903) provided an excellent résumé of the then existing state of our knowledge of the subject but made the error of considering the microspore mother cell as the first cell of the male gametophyte[3]. Since the gametophyte generation does not commence until the meiotic divisions are completed, the present account will begin with the tetrad stage.

THE MICROSPORE TETRAD

Microspores may be disposed in various ways in the tetrad. In the dicotyledons the arrangement is usually tetrahedral and in the monocotyledons it is isobilateral, but there are several exceptions in both groups. A decussate type is also not uncommon, having been recorded in *Magnolia* (Farr, 1918), *Atriplex* (Billings, 1934) and many other plants. On the other hand, a linear arrangement, which is of general occurrence in the case of megaspores, is extremely infrequent, and is found as a more or less regular feature only in some genera of the Asclepiadaceae (Gager, 1902) and in the genus *Halophila* of the Hydrocharitaceae (Kausik and Rao, 1942). Rarer still is the T-shaped tetrad, which has so far been reported only as an abnormality, as in *Apocynum* (Frye and Blodgett, 1905), *Aristolochia* (Samuelsson, 1914), *Butomopsis* (Johri, 1936*b*) and a few other plants. Of considerable interest are *Orchis* (Wille, 1886), *Neottia* (Goebel, 1933), *Nicolaia* (Boehm, 1931), *Musa* (Juliano and Alcala, 1933), *Agave* (Vignoli, 1936, 1937), *Typha* (Schaffner, 1897; Goebel, 1933) and *Habenaria* (Swamy, 1946*b*) in which two or three types of dispositions may be found in the same species. In *Zostera,* Hofmeister (1852) had thought that there is no tetrad formation, but this was a misinterpretation. Rosenberg (1901) showed that the elongated microspore mother cells, measuring 5 by 60 microns at the time of meiosis, divide in a plane parallel to the longitudinal axis of the cell and not transverse to it. The second division is also longitudinal, resulting in a group of four filiform cells which undergo further elongation and become approximately 8 by 2000 microns when mature[4].

---

[3] This mistake was later corrected by them (Coulter and Chamberlain, 1910) in their book entitled "Morphology of Gymnosperms".

[4] Filiform pollen grains are also known in *Phucagrostis major* (= *Cymodocea nodosa*) (Goebel, 1933), but in this case the exact sequence of development has not been studied and the elongation seems to take place after the reduction divisions are over.

Occasionally there are more, or fewer, than four spores per "tetrad". Wille in 1886 summarized the older literature and concluded that when there are two "microspores" this is due to a failure of the second division of the mother cell, and when there are three this is because the first division is unequal and the second takes place only in the larger of the two daughter cells. Five or more spores could sometimes arise as a result of subsequent division of one or more members of the tetrad. Many more instances, similar to those recorded by Wille, have been noted during recent years. To mention a few, Swamy (1946*a*) frequently found only two cells arising from the microspore mother cells of *Zeuxine;* Beer (1906) found six to ten in *Fuchsia;* Shoemaker (1926) noted six in a variety of apple called "Stayman Winesap"; and Penland (1923) saw as many as ten to twelve in *Rosa.* Similar reports have been made in *Atraphaxis* (Edman, 1931) and *Coffea* (Krug and Mendes, 1940). In some triploid varieties of *Musa* (Wilson, 1946) the "tetrad" has been found to consist of four large cells and a varying number of smaller ones, some of the latter being too minute to be counted accurately. In those cases where fewer than four cells are formed, it is due either to a failure of one division, or to the formation of "restitution nuclei", or to irregular wall formation. The last condition may give rise to one diploid and two haploid pollen grains (see Edman, 1931, Figs. 11–22; Afify, 1944, Fig. 8). When more than four cells are formed (polyspory), this is usually because of the occurrence of lagging chromosomes which organize into micronuclei and thus add to the total number of microspores. In general, however, such abnormalities are found only in hybrids, and the resulting pollen grains are usually non-functional.

A different type of abnormality, also appearing chiefly in hybrids and apomicts, is the frequent absence of wall formation during the meiotic divisions, followed by a fusion of the daughter nuclei (*e.g., Arnica alpina,* according to Afzelius, 1936). Such polyploid pollen grains often assume irregular shapes and a large size and may germinate prematurely, *i.e.,* before the division into tube and generative nuclei has taken place, as reported by Moffett (1932) in *Kniphofia.* In the pear variety "Beurre Bedford" (Thomas, 1942) the four microspore nuclei are able to complete this division, but the daughter nuclei frequently begin to fuse with one another, resulting in the formation of polyploid generative nuclei. In certain varieties of *Musa* (Dodds and Simmonds, 1946) the microspore mother cell becomes converted into a tetraploid microspore by a process of "double restitution" (see also Gentscheff, 1937, on *Hieracium*).

A polynucleate condition sometimes arises even by a fusion of the microspore mother cells, *i.e.*, by a process of cytomixis. In *Phleum pratense*, for instance, Levan (1941) found two haploid plants in which two to thirty microspore mother cells were observed to fuse in the meiotic prophase, giving rise to large plasmodia. In metaphase I these syncytes showed a single bipolar spindle on which all the bivalents had arranged themselves into one regular equatorial plate. A similar behavior has been reported by Stern (1946) in the microspore mother cells of *Trillium erectum* which had been suspended in sucrose media. The extent of fusions here appeared to be unlimited, although actually the maximum number of nuclei observed in a cell was 32. Cytomixis is also known to occur in later stages, *i.e.*, during or at the close of the meiotic divisions, as noted by Gelin (1934) in *Coreopsis tripteris*, and the multinucleate cells formed in this manner later break up into smaller units consisting of one or two nuclei each. It should be noted, however, that sometimes tapetal cells may also assume a similar appearance by becoming considerably enlarged at the expense of the adjoining cells and protruding inside the anther loculus. In *Atraphaxis frutescens* (Edman, 1931) they also develop an exine and thus complete the resemblance to real pollen grains, but are non-functional and soon degenerate.

Reference may also be made here to a peculiar abnormality noted in *Beta* (Levan, 1939) in which in a pocket of an anther loculus there were some pollen grains which were fifty times the normal size. They were irregular in shape and arranged in mosaic patterns. The origin of these peculiar structures could not be traced, but the phenomenon is attributed to a possible increase in the chromosome number or to a mutation.

FIRST DIVISION OF THE MICROSPORE

Usually the microspores soon separate from one another, but in certain cases they adhere in tetrads to form the so-called "compound pollen grains". As examples may be cited *Drimys, Anona, Mitriostigma, Sarcolaena, Jacquinia, Drosera, Typha, Fourcroya* and several members of the Bicornes, Epacridaceae, Juncaceae and Orchidaceae[5]. In the Mimosaceae there are larger units composed of 8 to 84 cells, and in a number of Asclepiadaceae and Orchidaceae-Ophrydeae all the microspores in a sporangium form a single mass called the "pollinium". These and other variations of external form have been known for a long time, and it is unnecessary to deal with them here, as they more appropriately belong to the general field of pollen morphology.

The newly formed microspore has a very dense cytoplasm (Fig. 1, A) but prior to its division the cell increases in volume and the accompanying vacuolation is followed by a displacement of the nucleus from its original central position to a place adjacent to the wall (Fig. 1, B). In most tropical plants it begins to divide almost immediately, but in plants of colder regions there is often a resting stage varying from a few days to several weeks. Finn (1937*a*), who made a recent study of this question, says that in the history of the male gametophyte overwintering may take place either at the uninucleate microspore stage or after the cutting off of the generative cell[6]. To mention a few instances, in *Tradescantia reflexa* (Sax and Edmonds, 1933) the resting period of the microspore is about four days or less; in *Styrax obassia* (Manshard, 1936) it is about a week; in *Himantoglossum hircinum* (Heusser, 1915) it is between two and three weeks; and in *Uvularia sessilifolia* (Alden, 1912), *Empetrum nigrum* (Samuelsson, 1913) and *Betula odorata* (Dahlgren, 1915) the microspores are said to pass the entire winter in the uninucleate stage.

The first division of the microspore (Fig. 1, C) gives rise to the tube (or vegetative) and generative cells (Fig. 1, D)[7]. Unlike the meiotic divisions, which occur more or less simultaneously in all the microspore mother cells of the anther (except in the case of very long anthers), the microspores usually divide without any such synchronization, and the same loculus may show different although not very widely separated stages of division and development. In those genera where the microspores remain together in a tetrad, all four cells are usually in the same stage of division, but not all the tetrads of an anther. As Barber (1942) says, a complete synchronization may be expected only where the microspores are united into pollinia (Mimosaceae, Asclepiadaceae, Orchidaceae), for here the cells probably exercise some influence over one another through the uncuticularized walls which lie between them. Exceptions do occur,

---

[5] In certain other plants also, where the microspores normally separate from one another, there may be some races in which they continue to adhere in tetrads. Levan (1942) has reported one such case in *Petunia nyctaginiflora*, and this peculiar behavior is attributed to a single recessive gene. Such an occurrence can be of value in the analysis of segregation, as has been done in some fungi and mosses, and it might be useful to find out whether it can be induced by the application of high temperatures or other environmental changes during and immediately after meiosis.

[6] Overwintering may also take place at the microspore mother cell stage before commencement of the reduction divisions (Finn, 1937*a*).

[7] In a heterozygous strain of *Antirrhinum majus*, Résende (1941) noted that many microspores, while still connected together into tetrads, already showed two or three "nuclei" instead of one; but these were not of the same size, one being larger and the other two smaller. The cause of the abnormality is not clear, and it seems possible that the supernumerary structures are unknown cytoplasmic granules of some kind rather than true nuclei.

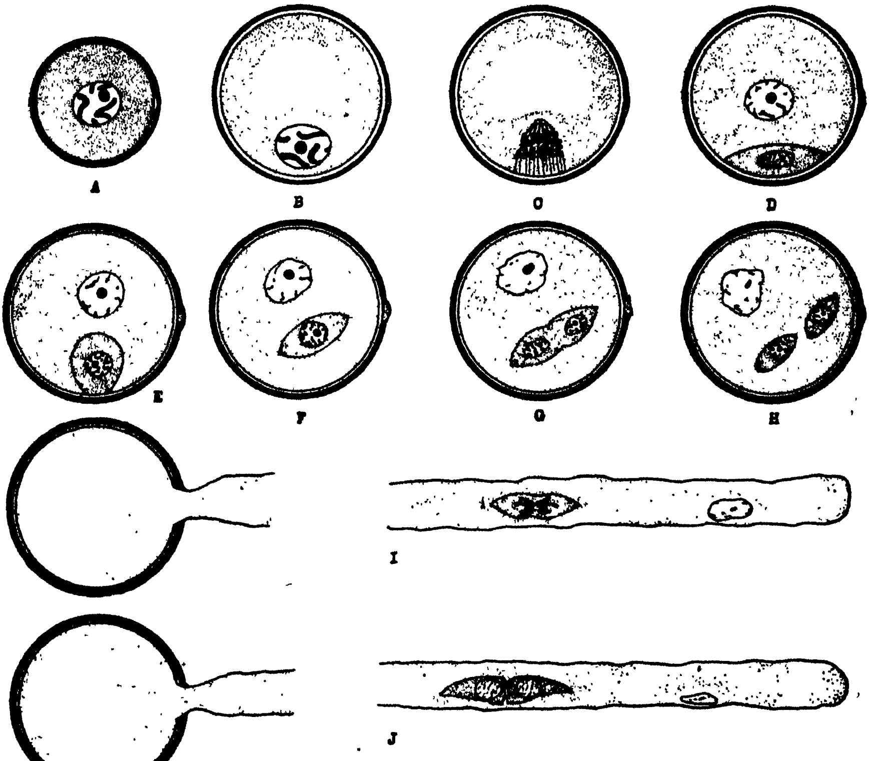


FIG. 1. Diagram showing development of male gametophyte in angiosperms. A. Microspore; B. Same, more advanced stage; C. Microspore nucleus dividing; D. Formation of vegetative (tube) and generative cells; E. Generative cell losing contact with wall of pollen grain; F. Generative cell imbedded inside vegetative cell; G, H. Division of generative cell to form male gametes; I, J. Same, division of generative cell taking place inside pollen tube.

however, even in such cases. For instance, Newman's (1934) Fig. 2 of the pollinium of *Acacia Baileyana* shows one of the microspores in prophase, another adjacent to it with the tube and generative cells already formed, and the rest in various intermediate stages.

A point of special interest in the division of the microspore nucleus is that the metaphase spindle usually shows a pronounced asymmetry, the wallward pole being blunt and the free pole acute[8]. Among recent workers who have studied this question, may be mentioned the names of Sax and Edmonds (1933), Geitler (1935), Darlington (1936), Hagerup (1938), Upcott (1939) and Brumfield (1941). The last-named author gives a full review of the previous literature on the subject, and his observations, which were made on smear preparations of *Allium, Pancratium* and *Tradescantia,* are summarized below.

According to Brumfield, the degree of asymmetry of the spindle is associated with the form of the prophase nucleus. In *Allium,* where the nucleus is strongly flattened on the wallward side of the microspore, the asymmetry is extreme; in *Pancratium,* where it is only slightly flattened, the asymmetry is of a lesser order; and *Tradescantia* shows an intermediate condition. The direct cause of the asymmetry is attributed to a difference in the time of development of the two spindle poles, the wallward pole developing more slowly than the other, presumably because of the smaller amount of cytoplasm associated with the former.

With the onset of the anaphase the asymmetry becomes less pronounced; for, the originally pointed vegetative pole now becomes somewhat broader in order to accommodate the daughter chromosomes belonging to this side, while the generative pole, which was at first rather diffuse and blunt, now tends to become more dense and acute, so that at late anaphase it is more nearly similar to the vegetative pole.

Brumfield adds that during the separation of the anaphase chromosomes there is also a "stretching" of the spindle, *i.e.,* an increase in its length accompanied by a decrease in the diameter. As the generative chromosomes are soon stopped by the wall, further elongation enhances the rate of movement of the vegetative chromosomes. "Thus the speed of the vegetative group is partly due to the movement of the chromosomes toward the pole, and partly due to the stretching of the spindle, while the speed of the generative group results only from its movement toward the pole".

---

[8] A similar conoidal or top-shaped spindle is also known to occur in the pollen grains of *Pinus* (Ferguson, 1904) and several other gymnosperms.

Hagerup's observations on *Orchis maculata* differ from those of Brumfield in that he found the metaphase spindles to be quite normal but those of anaphase and telophase to be asymmetrical. In the telophase the generative chromosomes are arranged in an almost plane surface parallel to the inner wall of the microspore, while the vegetative form a somewhat hemispherical pattern. This dissimilar grouping of the chromosomes also affects the behavior of the spindle fibers, which begin to disengage themselves first from the vegetative nucleus, although still remaining attached to the generative. The peripheral fibers, which are the first to be detached, at once spread laterally so as to surround the generative nucleus and form an "apparently fan-shaped figure". The cell-plate, which begins to be laid down at this stage, surrounds the generative nucleus like a hemispherical cap.

It must be noted, however, that the asymmetry of the spindle is not a universal feature. Thus, in *Asclepias* (Gager, 1902) and *Anthericum* (Geitler, 1935) both poles of the spindle are blunt; in *Adoxa* both are more or less pointed (Lagerberg, 1909); and in *Podophyllum* (Darlington, 1936) both asymmetrical as well as symmetrical division figures are said to occur in the same loculus. Further, the spindle may not always be situated near the wall of the pollen grain. In *Myricaria* (Frisendahl, 1912), *Adoxa* (Lagerberg, 1909), *Sambucus* (Schürhoff, 1921), *Cotylanthera* (Oehler, 1927), *Cuscuta* (Johri, 1934) and *Uvularia* (Geitler, 1935) it occupies almost the entire width of the grain; nevertheless, the two cells formed by the division are always unequal[9]. In the first-named instance this is said to be brought about by a contraction of the spindle towards one side, but, as remarked by Geitler (1935), this point needs confirmation.

THE GENERATIVE CELL

Goebel (1933) believed that in the angiosperms the generative cell is cut off on the distal or ventral side of the microspore. Geitler

---

[9] In the very few instances where equal daughter cells have been reported after the first division of the microspore, as in *Cuscuta epithymum* (Fedortschuk, 1931) and *Strychnos sansibariensis* (Mohrbutter, 1936), this seems to be an abnormality leading to the formation of double microspores, each of which would probably divide again to give rise to the tube and generative cells.

(1935) has shown, however, that this is not always the case and that it may be cut off either (*a*) on the outer side, (*b*) on the inner side, (*c*) on a radial wall, or (*d*) in a corner instead of the middle of the radial wall. To cite a few instances, the first-named condition is known to occur in *Elodea* (Wylie, 1904), *Vaccinium* (Samuelsson, 1913), *Albizzia* (Maheshwari, 1931), *Acacia* (Newman, 1934), *Asimina* (Locke, 1936), *Ludwigia* (Geitler, 1937) and most members of the Orchidaceae (Swamy, 1946*b*); the second in *Symplocarpus* (Duggar, 1900), *Xyris* (Weinzieher, 1914), *Tradescantia* (Sax and Edmonds, 1933), *Apocynum* (Müller, 1936), *Erica* (Geitler, 1935), *Uvularia, Narcissus, Bulbine, Gasteria, Aloe, Clivia* (Geitler, 1935), *Juncus* (Wulff, 1939*a*), *Myristica* (Joshi, 1946), and most members of the Cyperaceae (Piech, 1924*a, b,* 1928; Tanaka, 1939*a, b,* 1940*a,* 1941)[10]; the third in *Allium* (Geitler, 1935); and the fourth in *Lilium* (Strasburger, 1908), *Anthericum, Convallaria* and *Ruscus* (Geitler, 1935)[11].

Geitler (1935) believes that whatever the position may be, it is a constant one in individuals of the same species and sometimes in all the species of a genus or family[12] and that it is not influenced either by the mode of quadripartition of the microspore mother cell (whether simultaneous or successive) or by the contents of the anther loculus. Among possible exceptions to this rule may be cited the case of *Adoxa,* in which, according to Lagerberg (1909), the generative cell may be formed on any side of the microspore. Geitler (1935) contradicts it, however; according to him, Lagerberg was probably misled by the fact that the nearly isodiametric pollen grains of this plant often appear so deformed in microtome sections of fixed material that it is difficult to recognize their orientation. A second instance is that of *Periploca sepium,* in which Sax and Husted (1936) report that "the axes of the nuclear division in the microspores are not constant in respect to the axes of either the first or second meiotic divisions, and although there is a tendency for the generative nuclei to lie nearer the outer wall of the microspores, they may be found in any position. . . . Either internal or external environment may disturb polarity and the regular development of the microspore". While this may be true, the illustrations (Figs. 19–21) presented by the authors are not convincing, for in every case figured by them the generative cell is already free from the wall of the microspore and there is no certainty that it had not moved away from its original position in the cell. A change of position of the generative cell is of such frequent occurrence that considerably earlier stages (before the cell has lost contact with the wall of the microspore) must be sought in order to be sure of the place where it is cut off.

---

[10] The only exception is the genus *Rhyncospora* in which the generative cell is cut off on the inner side (Tanaka, 1941).

[11] The particular angle in which the generative cell is cut off has not been determined in these cases.

[12] The precise position in which the generative cell is cut off can, of course, be recognized most clearly only in those plants where the pollen grains remain together in tetrads, but even when they soon fall apart the furrows and germ-pores often serve as useful guides in determining it. There still remains, however, a large number of genera in which the pollen grains round up at such an early stage that it becomes impossible to distinguish one side from the other.

Although at first lenticular, the generative cell may frequently change its form after it has moved away from the wall of the microspore and comes to lie inside the vegetative cytoplasm[18]. Most commonly it is elliptical or spindle-shaped (Fig. 1, F), less frequently sickle-shaped. In *Campanula ranunculoides* (Schnarf, 1937) the two ends of the cell are dissimilar, one being pointed and looking like a "tail" and the other more or less blunt and swollen so as to look like a "head". In some cases the cell becomes so long as to occupy the entire width of the pollen grain, coming quite close to the inner wall of the latter on either side: *Cuscuta* (Finn, 1937*b*), *Ottelia* (Narasimha Murty, 1935), *Lactuca* (Jones, 1927). In *Monochoria* (Banerji and Haldar, 1942, Fig. 18) it is fully one and a half times as long as the diameter of the pollen grain and is accommodated in the latter only by the bending in of its whip-like ends. In *Erythronium* (Schaffner, 1901) the generative cell is said to be amoeboid and may assume peculiar shapes. Several other recorded instances of a change in its form must, however, be interpreted differently, for a spindle-shaped generative cell may appear round when cut across and oval when cut obliquely. Frye (1902) wrongly thought that such appearances in *Asclepias* are due to a real change in the shape of the cell (see comments by Finn, 1925).

As emphasized in several recent papers (Finn, 1935; Wulff and Maheshwari, 1938), the generative nucleus is not naked but has an investment of its own cytoplasm which is distinguishable from the vegetative cytoplasm by its hyaline appearance. Even with the use of the simple and quick acetocarmine technique, Schnarf (1937) was able to demonstrate the generative cytoplasm in several species in which previously only a naked nucleus had been reported, and according to Finn (1935) all reports of the occurrence of a naked nucleus are to be attributed to inadequate technique. For satisfactory observations he recommends fixation in Nawaschin's fluid and staining with iron-haematoxylin followed by aqueous erythrosin. With this method the red-stained cytoplasm of the generative cell often projects quite distinctly on the somewhat blue background of the vegetative cytoplasm. Another method suggested by him is the use of the Pianese stain[14] which gives especially clear views of the cytoplasmic layer surrounding the generative nucleus or sperm nuclei, provided of course that a proper differentiation has been obtained[15].

---

[18] The gradual extension of the vegetative cytoplasm around the generative cell and the consequent "engulfing" of the latter has been described by several workers, *viz.*, Friemann (1910), Wefelscheid (1911) and Capoor (1937*a*).

Although usually thin, the cytoplasmic layer may sometimes be quite prominent and show special structures inside it. Ruhland and Wetzel (1924) and Krupko (1926) noted the existence of plastids in the generative cell of certain angiosperms, and recently Suita (1938) has reported small "droplets" resembling chondriosomes in the living pollen grains of *Crinum* and *Hippeastrum*. A vacuome has also been demonstrated in the generative cell of several monocotyledons (Wulff, 1933)[16], a particularly good instance of this kind having been reported recently in *Hemerocallis tubiflora* (Beck and Joly, 1941)[17]. Of special interest are the studies on living pollen grains and pollen tubes of *Narcissus, Asclepias, Vinca, Crinum* and *Lilium* conducted in Finn's laboratory (Kostriukova and Benetskaia, 1939; Kostriukova, 1939*a, b;* Benetskaia, 1939), which have confirmed the presence of mitochondria and vacuoles in the generative as well as in the sperm cells of several species of angiosperm (see also Anderson, 1939).

---

[14] This consists of malachyte green, fuchsin S, nigrosin, a saturated alcoholic solution of copper acetate, and water (Faworsky, 1906). See also Maheshwari and Wulff (1937) for a review of the methods used in the study of the male gametophyte of angiosperms.

[15] Dr. L. E. Anderson (Duke University) informs the author that a still more reliable and irrefutable method, where possible, is the study of living material. By careful manipulation of light, the cytoplasmic sheath around the generative and male nuclei was clearly seen in living unstained pollen tubes of several species studied in his laboratory.

[16] Piech (1924*a*) expressed the view, now shown to be incorrect, that the generative plasm consisted only of proteids and did not contain any osmotically active materials.

[17] The generative cell of this species has an orange-red color, but the pigment is located in the nucleus instead of the cytoplasm (Beck and Joly, 1941).

DIVISION OF THE GENERATIVE CELL

The generative cell may divide either in the pollen grain (Fig. 1, G, H) or in the pollen tube (Fig. 1, I, J). Formerly, the latter condition was believed to be the more frequent. While this may still be true, the large number of genera which have since been found to have three-celled pollen grains indicates that this condition is by no means uncommon (see Schnarf, 1939), and it seems certain that many of the older records were based on a study of immature pollen[18]. To mention a few instances, Carlson and Stuart (1936) reported two-nucleate pollen grains in certain species of *Salvia,* but Schnarf (1937), Finn (1939) and Stejskal-Streit (1940) have shown that the development continues up to the three-celled stage. Jüssen (1928) reported binucleate pollen grains in *Calla,* but Dudley (1937) has found several instances in which the generative nucleus had already divided when the pollen grains were still in the anther. Fedortschuk (1931) and Negodi (1936) reported two-nucleate pollen grains in *Cuscuta monogyna* and *Bellis perennis,* respectively, but Finn (1937*b*) and Schnarf (1937) have shown them to be three-nucleate[19].

Occasionally both two- and three-celled pollen grains have been reported in the same species, as in the cleistogamous flowers of *Viola* (West, 1930), in *Nicotiana* (Poddubnaja-Arnoldi, 1936), *Epimedium* and *Iris* (Schnarf, 1937), but this is probably due to environmental effects. For instance, Poddubnaja-Arnoldi (1936) found that in several kinds of pollen grains, which are normally shed at the binucleate stage, the generative nucleus could be made to divide before germination if the grains were allowed to lie for a time on a sugar-agar substrate. Eigsti (1941) was similarly able to induce a precocious division of the generative cell of *Polygonatum canaliculatum*. An especially interesting case is that of *Holoptelea integrifolia* (Capoor, 1937*b*), in which the pollen grains are shed at the two-celled stage but the generative cell divides on the surface of the stigma before the pollen tube has started to grow. This is comparable to the condition in some conifers like *Cupressus, Juniperus, Taxus* and *Cunninghamia,* where the division of the microspore nucleus occurs on the nucellus.

---

[18] It is of interest to note that as the pollen grain grows older, the vacuoles become smaller and more evenly distributed; finally they seem to disappear almost entirely, so that the mature pollen grain, like the young microspore, again shows a dense cytoplasm devoid of all conspicuous vacuolation. This is such a constant feature in most angiosperms, excluding some aquatics (see Wulff, 1939*b*), that it serves as a useful check for judging whether the pollen grain is fully mature or not (see Schnarf, 1937).

[19] Mention may also be made here of a few reports of the shedding of uninucleate pollen grains in which the microspore nucleus is still undivided: *Argemone mexicana* (Bose, 1937), *Carthamus tinctorius* (Banerji, 1940), *Crotalaria juncea* (Banerji and Samal, 1936), *Corchorus olitorius* (Banerji, 1933), *Leandra cordifolia* (Subramanyam, 1942), *Pennisetum typhoideum* (Rangasami, 1935), *Putranjiva Roxburghii* (Dutt, 1943), *Santalum album* (Srinivasa Iyengar, 1937) and *Sesamum indicum* (Nohara, 1934). My own studies show that the pollen grains of *Corchorus olitorius, C. capsularis, C. acutangulus* and *Crotalaria juncea* are shed at the two-celled stage, and of *Carthamus tinctorius* at the three-celled stage. In *Carthamus* even acetocarmine preparations clearly showed the two elongated male nuclei, such as are characteristic of other members of the Compositae. The remaining instances deserve reinvestigation.

There is considerable evidence to indicate that even in those plants where the pollen grains are shed in the two-celled condition, the generative nucleus is already in the prophase stage and the process of division is merely continued on the stigma or in the style. Sometimes it may even show a prometaphase stage[20], which is distinguishable from a typical metaphase only by the presence of a nuclear membrane and the absence of a spindle. This has been clearly demonstrated in *Impatiens* (Wulff, 1934; Heitz and Résende, 1936), *Bulbine* (Geitler, 1942) and in other plants.

Further details of the division vary according to whether it takes place in the pollen grain or in the pollen tube. In the former case spindle fibers and a normal metaphase have been regularly observed (see especially Finn, 1925, on *Asclepias* and *Vinca*) and the division does not seem to differ in any essential way from a normal mitosis. Regarding the mechanism of cytokinesis, a regular cell-plate has been recorded in *Ruppia* (Murbeck, 1902; Graves, 1908), *Asclepias* (Finn, 1925), *Echinops* (Poddubnaja-Arnoldi, 1927), *Portulaca* (Cooper, 1935*b*) and in other plants. In *Echeveria* (Harrig, 1919), *Scirpus* (Piech, 1924*b*), *Vinca* (Finn, 1928*a*), *Cuscuta* (Fedortschuk, 1931), *Halophila* (Kausik and Rao, 1942) and *Vallisneria* (Wylie, 1923), on the other hand, partition of the cell takes place by means of a constriction, and even if a cell-plate is laid down it soon becomes obliterated. Witmer (1937) observed cell-plates as well as constriction furrows in *Vallisneria,* but these two factors were found to vary in importance, and it is concluded that either of them can be entirely excluded from the process of division. In certain instances a definite cell-plate is distinguishable, but it soon fades away leaving the final separation of the sperm cells to a constriction which arises shortly afterwards. In others the cell-plate is more persistent, and the progress of the constriction is delayed in this region, although evident on either side of it. In still other instances the splitting of the cell-plate separates the sperms before the constriction has made much progress.

---

[20] Marquardt and Ernst (1939/40) hold a different opinion, but see criticism by Geitler (1942).

The recent improved methods of making permanent mounts of pollen tubes have given a considerable stimulus to studies on the mechanism of division of the generative cell when it takes place in the tube. There is, however, no unanimity of opinion about the details of the process, and widely different interpretations have sometimes been given on one and the same genus and species. The chief points to be considered are: whether a regular metaphase plate is formed during the division; whether spindle fibers are present or absent; and whether cytokinesis takes place by constriction or by cell-plate formation.

Nawaschin (1910) in *Lilium Martagon,* O'Mara (1933) in *L. regale*[21], Wulff and Raghavan (1937) in *Nemophila insignis,* Botschanzeva (1937) in *Tulipa,* Raghavan *et al.* (1939) in *Impatiens balsamina,* and a number of older workers were unable to recognize any regular metaphase plate. On the other hand, Cooper (1936) has reported their occurrence to be a regular feature in *Lilium regale, L. auratum* and *L. philippinense,* and believes that O'Mara's (1933) figures of an "irregular metaphase" really represent late prophases, the true metaphase having been missed by him. Upcott (1936), in six species of *Tulipa,* and Madge (1936), in *Hedychium gardnerianum,* also found a metaphase plate, the only important difference being its oblique orientation which gives more space to the chromosomes for their proper alignment. More recently, well differentiated metaphase plates have been recorded in *Eichhornia* (Banerji and Gangulee, 1937), *Tulipa, Amaryllis, Nicotiana, Forsythia, Camellia, Bryophyllum* (Johnston, 1941) and *Eschscholtzia* (Beatty, 1943).

---

[21] See, however, his Fig. 9.

Certain other workers have reported varying results. Thus, Trankowsky (1931, 1939) found a regular metaphase plate in *Hemerocallis* and *Lathyrus,* in which the pollen tubes are appreciably broader than the diameter of the metaphase spindle, but not in *Galanthus, Convallaria, Delphinium* or *Scilla* which have narrower tubes. Sax and O'Mara (1941) state that the division is more or less normal in *Scilla, Bellevalia* and *Zephyranthes,* but atypical mitoses are frequent in *Tradescantia, Gasteria* and *Lilium.* They conclude that when spatial relations are adequate, the chromosomes form an equatorial plate and separate normally in the metaphase; but large chromosomes in a narrow pollen tube divide without the formation of an equatorial plate, and the daughter chromosomes appear to act as independent units passing irregularly to the poles.

A careful study of this conflicting mass of evidence seems to indicate that, in general, a metaphase plate is organized, but a narrow pollen tube often limits its width, and large chromosomes may be unable to space themselves as they would in an unconfined mitosis, so that the entire body of a chromosome lies at right angles to the plate. There is evidence, however, that even in these cases the fundamental features of a metaphase, *i.e.,* an equatorial alignment of the kinetochores, is still present (Eigsti, 1940).

Regarding the presence or absence of spindle fibers, Nawaschin (1910) in *Lilium Martagon,* Welsford (1914) in *L. auratum* and *L. Martagon,* Trankowsky (1931) in *Convallaria majalis* and *Galanthus nivalis,* O'Mara (1933) in *Lilium regale,* Hoare (1934) in *Scilla nonscripta*[22], Fuchs (1936) in *Elaeagnus angustifolius,* Wunderlich (1937) in *Muscari racemosum* and *M. comosum,* Finn (1939) in *Phlomis tuberosa,* Raghavan *et al.* (1939) in *Impatiens balsamina,* and several other workers failed to find a spindle[23]. On the other hand, Trankowsky (1931) in *Hemerocallis fulva*[24], Cooper

---

[22] Hoare (1934) notes, however, that in his material spindle fibers were not clearly defined, even in normal mitoses, and that the distinctness of the spindle varied with the type of fixative employed and with the rapidity of its penetration.

[23] Sax and O'Mara (1941) also did not observe any "well defined spindle mechanism" in their preparations of *Lilium,* but they admit having made no attempt "to differentiate such structures with special fixing and staining techniques".

[24] This difference between *Convallaria* and *Galanthus* on the one hand and *Hemerocallis* on the other is attributed by Trankowsky (1931) to the "Raumverhältnisse" of the generative cell and the pollen tube [see also a subsequent paper by the same author (Trankowsky, 1939)].

(1936) in *Lilium auratum, L. regale* and *L. philippinense,* Madge (1936) in *Hedychium gardnerianum,* Upcott (1936) in *Tulipa,* Eigsti (1939) in *Lilium canadense, L. speciosum, L. auratum, Polygonatum commutatum, Convallaria majalis* and *Tradescantia reflexa,* Johnston (1941) in *Tulipa Gesneriana, Amaryllis* spp., *Nicotiana tabacum, Forsythia viridissima, Camellia japonica* and *Bryophyllum pinnatum,* and Beatty (1943) in *Eschscholtzia californica* have emphasized that spindle fibers are present and function in the same way as in normal mitosis.

Wulff (1935) reported that the pollen tubes of *Narthecium ossifragum,* grown in culture, are five to six times as broad as those growing in the style. He believed this difference in width to be a factor which affected the mechanism of the division in an important manner. The stylar pollen tubes, being very narrow, did not show spindle fibers or metaphase plates, whereas cultured pollen tubes, being broader, showed both of these formations. However, other workers, *e.g.,* Cooper (1936) and Eigsti (1939), have not been able to confirm this difference, and Beatty (1943) has shown that in *Eschscholtzia californica* the width of the pollen tubes may vary in culture as well as in stylar tissue. In artificial cultures also the tubes are narrower if the sugar concentration is lowered.

Johnston (1941) rightly suggests that this divergence of opinion is partly due to the difference in staining methods employed by different workers and to the fact that an intensity of staining which is adequate or just right for the chromosomes often fails to bring out the spindle fibers which are more clearly seen in overstained material. Delafield's haematoxylin is superior to Heidenhain's haematoxylin for this purpose and should always be used for comparison. In addition, there is also some indirect evidence of the presence of the spindle, for during the anaphase stage chromosomes with terminal attachment points appear as straight rods and those with median attachment points in the form of a J or V—a condition which corresponds with that in ordinary mitosis. Further, Suita (1939*a*) and Eigsti (1940) have found that this division reacts to colchicine treatment in precisely the same way as ordinary mitosis. Now, if the colchicine-effect occurs through an action on the spindle mechanism, as is generally agreed at the present time, it seems logical to infer that this mitosis does not differ from other mitotic divisions regarding the presence of spindle fibers.

Dealing finally with the mode of cytokinesis[25], Raghavan *et al.* (1939) in *Impatiens balsamina,* Banerji and Gangulee (1937) in *Eichhornia crassipes,* and several other authors report that the division of the generative cell occurs by a constriction. Eigsti (1940) also states that cell-plates are "difficult to find and are of rare occurrence; furthermore, their presence does not necessarily imply functional relationships. They are doubtless temporary structures, or they would have been found more frequently in the various slides". On the other hand, Cooper (1936) found a clear cell-plate in *Lilium regale,* and this has also been confirmed from studies on living pollen tubes of *Crinum Hildebrandtii* (Kostriukova, 1939*a*), *Lilium Martagon* (Kostriukova, 1939*b*) and *Narcissus poeticus* (Kostriukova and Benetskaia, 1939). The most extensive work is that of Johnston (1941) who points out that the inability to see cell-plates is probably due to the exclusive use of Heidenhain's haematoxylin. With Delafield's haematoxylin he was able to find a clear cell-plate, appearing as a darkly staining continuous structure, in every species studied by him, viz., *Tulipa Gesneriana, Amaryllis* sp., *Nicotiana tabacum, Forsythia viridissima, Camellia japonica* and *Bryophyllum pinnatum.* Regarding the mechanism of separation of the male gametes, he says that this is characterized by the appearance of certain lightly staining areas on either side of the cell-plate. These soon become vacuolate and later fuse into a continuous hyaline zone. New plasma membranes now form in contact with the outer margins of the hyaline areas, and the gametes round up, leaving the cell-plate as a line of cleavage or separation.

It may therefore be concluded that as a rule the division of the generative cell, whether it takes place in the pollen grain or in the pollen tube, occurs in a fairly normal manner. Only in such cases where the tube is very narrow and the chromosomes rather large, may be there some disturbance of the metaphasic alignment, resulting in their crowding or buckling, or an oblique arrangement. It seems that in many cases the metaphase stage has been missed, due to its very short duration, and one may be misled to imagine that the nucleus passed directly from the prophase into the anaphase. Finally, the division of the generative cell may take place either by means of constriction furrows or by the laying down of a cell-plate.

---

[25] There are also occasional reports of a failure of cell division following the nuclear division inside the generative cell. Thus, Nawaschin and Finn (1913) in *Juglans,* and Tschernojarow (1926) in *Myosurus,* report that the generative cell remains binucleate, even up to the time of discharge of the two nuclei in the embryo sac. Tschernojarow's figures seem to indicate that a constriction furrow starts to form and the generative cell becomes narrowed in the mid-region, but the division is not completed.

THE MALE GAMETES

Formerly it was believed that whenever the division of the generative cell occurs in the pollen grain it is followed by the formation of sperm cells, but that if it takes place in the streaming cytoplasm of the pollen tube only nuclei are formed (see Dahlgren, 1927*a;* Schnarf, 1929). More precise observations made during recent years have shown, however, that under either condition the male gametes are definite cells and that they retain their cytoplasmic sheaths throughout their course in the pollen tube.

Considering first the case of *Lilium,* which has been a favorite object for such studies, Guignard (1889) figured the male gametes of *L. Martagon* as cells, but Körnicke (1906), Strasburger (1908) and Nawaschin (1910) thought that the cytoplasmic sheath is lost during division of the generative cell so as to leave the male nuclei naked in the cytoplasm of the pollen tube. Regarding the exact time of disappearance of the sheath, however, these authors had no agreement among themselves. According to Körnicke, it is lost when the generative cell is in the prophase; according to Strasburger, in the metaphase; and according to Nawaschin, only in the telophase. A few years later, Welsford (1914) and O'Mara (1933) made a more detailed study and showed that in both *L. Martagon* and *L. auratum* definite sperm cells are formed. O'Mara believed that eventually the cytoplasm dissolves away so that only the naked nuclei enter the embryo sac. Cooper (1936), working with *L. Martagon, L. auratum* and *L. philippinense,* showed, however, that the male gametes persist as cells right up to the time they reach the embryo sac. This has since been confirmed by Anderson (1939) who adds that the cytoplasmic sheaths around the male nuclei possess all the inclusions which are normally present in the vegetative cytoplasm. Mitochondria are abundant, as well as transitional stages between them and plastids. The failure of other workers to see the cytoplasm is considered to be due at least partially to their use of staining techniques which, although suitable for nuclear studies, fail to bring out the cytoplasm to the best advantage. With Heidenhain's haematoxylin, when the pollen tubes are destained to show nuclear structures, the stain is already too faint to show the cytoplasm around the nuclei.

The cytoplasmic sheath around the male nuclei has also been observed in plants other than *Lilium.* As examples may be cited *Juglans regia* and *J. nigra* (Nawaschin and Finn, 1912)[26], *Myosurus* (Tschernojarow, 1926), *Plumbagella* (Dahlgren, 1916) and *Oenothera* (Ishikawa, 1918). Finn (1935, 1940, 1941), who has been the most active worker in the field, definitely states that the occurrence of male cells may be assumed in all angiosperms and predicts that in those plants where only naked nuclei have been reported, proper methods of fixing and staining will eventually reveal a thin layer of lighter staining cytoplasm around them (see also Kostriukova, 1939*a, b,* 1941, 1945; Benetskaia, 1939; and Kostriukova and Benetskaia, 1939).

The male gametes show a range in shape similar to that of the generative cell. Although usually oval or elliptical, sometimes they are lenticular, spindle-shaped or vermiform[27]. In the Compositae they are usually considerably elongated, and in some grasses like *Oryza sativa* (Nagouchi, 1929) they are spiral-shaped. In *Leycesteria formosa,* belonging to the Caprifoliaceae (Schnarf, 1937), one end may be pointed and the other blunt. This feature is particularly pronounced in the male cells of *Asclepias cornuti* (Finn, 1925), one end of which is nearly spherical, while the other forms a tail-like projection. In *Elodea* (Wylie, 1904), *Ulmus* (Shattuck, 1905) and *Vallisneria* (Wylie, 1923) the two male cells are connected by means of a narrow bridge and remain together throughout their journey in the pollen tube.

Sometimes the male gametes may change their shapes either during their passage to the embryo sac or after their discharge into it. In his well known work on double fertilization, Nawaschin (1898) called attention to the fact that in *Lilium* and *Fritillaria* the sperms lose their worm-like form just before fertilization. Shibata (1902) noted that in *Monotropa* they are elongated and slightly curved in the beginning but become rounded afterwards. In *Silphium* (Land, 1900), *Fritillaria* (Sax, 1916), *Taraxacum* (Poddubnaja-Arnoldi and Dianowa, 1934) and *Lactuca* (Jones, 1927) also they are at first elongated but become shorter and thicker on their approach to the female nuclei. In *Juglans* (Nawaschin, 1900), on the other hand, they are rounded in the beginning but later become more or less banana-shaped; and in *Tulipa* (Botschanzeva, 1937) they assume a vermiform outline. In *Vallisneria* (Wylie, 1941) they emerge from the pollen grain in a somewhat contracted form and are almost isodiametric during their progress through the short style. As the pollen tube enters the ovarian cavity, however, they become considerably elongated, but again present a rounded appearance at the time of their discharge into the embryo sac. Gerassimova (1933) gives an especially detailed account of the changes undergone by the male gametes of *Crepis* and remarks that they are so rapid as to make it impossible to describe the shape in one word. She considers these alterations to be stages in the final "maturation" of the sperms before they are ready to unite with the female nuclei. In a recent work on *Epipactis,* Hagerup (1945) states: "The male nuclei were long and narrow when they were in the pollen tube, where there was very little room. But as soon as they enter the embryo sac, where there is plenty of room, they at once change their form, become short, discoid, and usually furnished with 1–3 angles".

---

[26] Nawaschin and Finn (1912) figured a clear space around the male nuclei of *Juglans.* Finn (1925) explained later that this space represents the cytoplasmic sheath around each nucleus.

[27] Guilliermond, Mangenot and Plantefol (1932) state that, as a rule, the male gametes are vermiform and spiral. As Newman (1934) has already pointed out, this statement is not correct, for, although the vermiform type is certainly characteristic of some members of the Liliaceae and Compositae, the spherical or elliptical type is, on the whole, more widely distributed in other angiosperms.

Usually both the male gametes from a single pollen grain or pollen tube are of similar size and shape and apparently quite indistinguishable from each other. Guignard (1899) positively stated, however, that in *Endymion mutans* (= *Scilla nonscripta*) the male nucleus which fertilizes the egg is smaller than the one fusing with the polar nuclei, and Hoare (1934) has confirmed this, although admitting that the size difference is not a regular feature. Blackman and Welsford (1913) in *Lilium Martagon,* Sawyer (1917) in *Iris versicolor,* Sax (1916) in *Fritillaria pudica,* Nothnagel (1918) in *Trillium grandiflorum,* Newman (1934) in *Acacia Baileyana,* Johansen (1936) in three species of *Fouquieria,* Raghavan (1937) in *Cleome chelidonii,* and Smith (1942) in *Camassia leichtlinii* have also reported that the male gamete fertilizing the egg cell is somewhat smaller than the one fusing with the polar nuclei. Gurgenova (1928) observed that in *Phelipaea ramosa* (= *Orobanche ramosa*) a difference was recognizable even in the pollen tube, the nucleus destined to fuse with the egg staining less intensively than the other; and Rudenko (1930) has stated that in *Lathraea squamaria* the former is spherical and the latter elliptical.

In certain other instances, however, the reverse condition has been reported. Persidsky (1926) states that in *Orobanche cumana* and *O. ramosa* the sperm nucleus fertilizing the egg is shaped like a hemisphere and is larger than the other which has an oval outline. According to Finn and Rudenko (1930) also, in *O. ramosa* the sperm nucleus fusing with the egg is larger or looser and more faintly staining than the other. In *Vallisneria* (Wylie, 1923) both the male gametes are oval at the time of their emergence from the pollen tube, but the second soon becomes spherical. Very recently Kadry (1946) has reported that in *Cardiospermum* the male nucleus fertilizing the egg is thick and rounded in the front and tapering behind, and its length is about four times the diameter of the other nucleus which is more or less spherical[28]. In *Vinca minor* (Finn, 1928*a*) also, the sperm cells are unequal, one with a larger and the other with a shorter plasma tail, but it could not be determined as to which of them fuses with the egg and which with the polar nuclei.

There are very few observations on the precise nuclear condition in which the male gametes participate in fertilization. In most instances the nuclei have been observed to be in a resting condition. As examples may be cited: *Cypripedium* (Pace, 1907—the author adds, however, that it is not uncommon to find separate "spiremes" already well advanced before the membrane between the fusing nuclei breaks down), *Oenothera* (Ishikawa, 1918), *Vallisneria* (Wylie, 1923), *Trillium* (Nothnagel, 1918), *Phaseolus* (Weinstein,

---

[28] In reports of this nature, due care has, of course, to be taken that the observed differences are not due to the plane of sectioning, for the same nucleus or cell may appear small and rounded if cut across, elongated if cut longitudinally, and oval or elliptical if cut obliquely. Stages in fertilization are only rarely seen, and it is possible that in any given section one male gamete may have been cut across its longer diameter and the other in a plane at right angles to it so that the two appear to be of different sizes although really quite similar. Also, as pointed out already, the male gametes often change their form, and, as Gerassimova (1933) has suggested, the recorded size differences may well be due to a "different tempo" in their transformation and maturation. Considered in this light, therefore, the differences that have been reported in the size and shape of the two male gametes released by a single pollen tube may be regarded as variations which are of no special significance.

1926), *Viola* (West, 1930), *Cleome* (Raghavan, 1937), *Utricularia* (Kausik, 1938) and some forms of *Malus* (Wanscher, 1939). In certain other plants they have been seen in the prophase: *Lilium Martagon* and *L. auratum* (Welsford, 1914), *Fritillaria pudica* (Sax, 1918), *Lilium philadelphicum* and *L. longiflorum* (Weniger, 1918), *Viola odorata* (Madge, 1929), *Tulipa* (Botschanzeva, 1937) and *Cyanotis cristata* (Lakshminarasimha Murty, 1938). In the cleistogamous flowers of *Viola* (Madge, 1929) the male nucleus is in a "spireme" when it comes in contact with the egg nucleus. In *Triticum durum hordeiforme* (Sax, 1918) the first male nucleus and the nucleus of the egg were both found to be in the "spireme" stage, but the second male nucleus had already progressed into the early metaphase condition at the time of its fusion with the polar nuclei.

According to some reports, the fusion may take place even before the male nuclei have recovered from their original telophasic condition. Nawaschin (1910) believed this to be the case in *Lilium Martagon,* and Frisendahl (1912) in *Myricaria germanica.* Also in *Scilla nonscripta,* according to Hoare (1934), the male nuclei formed after division of the generative cell "never pass into a completely resting stage" but show a dense chromatin reticulum which is interpreted as "the previous telophase"; and in *Acacia Baileyana* (Newman, 1934) "although the nuclei are in a telophasic condition, the second male nucleus has time to proceed further into the resting condition than the first".

There are conflicting reports regarding the occurrence of a nucleolus in the sperm nuclei. In certain plants, *e.g.*, *Cuscuta* (Finn, 1937*b*), *Peltandra* (Goldberg, 1941) and *Camassia* (Smith, 1942), no nucleolus has been observed. In *Orobanche* (Finn and Rudenko, 1930) and *Crepis* (Gerassimova, 1933) a nucleolus was not distinguishable at the time when the sperm cells were first formed, but it appeared shortly before fertilization, and in *Taraxacum kok-saghys* (Warmke, 1943) it was seen only after fertilization. In *Viola riviniana* (West, 1930), on the other hand, the male nuclei always show a distinct nucleolus, and the one entering into triple fusion is "represented almost entirely by its nucleolus".

Coming finally to the question of the movement of the generative cell and the male gametes, it is well known that the sperm cells of many thallophytes and of all bryophytes and pteridophytes are actively motile. Even among the gymnosperms, the Cycadales and Ginkgoales have ciliated sperms, and the pollen tube serves as a haustorial organ rather than as a device for conveying them to the archegonia. In the Coniferales, Gnetales and angiosperms the situation seems to be different, for here the cilia are lost and the pollen tube becomes the agent for transport of the male gametes from the pollen chamber or the stigma to the embryo sac. Long ago Strasburger (1884, 1908) put forth the view that this transport is accomplished passively by the streaming movements of the vegetative cytoplasm. Nawaschin (1898, 1909), on the other hand, considered that the vermiform appearance of the male nuclei, observed in several members of the Compositae and Liliaceae, indicates that they have the power of independent movement. Subsequent authors have, on the whole, been in agreement with Strasburger's view. A few who came to a different conclusion have expressed themselves in a very guarded manner. Thus, West (1930) says: "the male nuclei in *Viola Riviniana* never become vermiform, and there is therefore nothing to suggest that they are capable of movement. At the same time, the form of the whole male cell certainly indicates motility. . . ." In a work on *Scilla non-scripta,* Hoare (1934) comments as follows: "The vermiform shape assumed by the male nuclei is suggestive of independent movement through the cytoplasm, but this shape would also be the one which would offer the least resistance to its carriage by cytoplasmic currents. As the sperm applies itself to the egg nucleus, it coils closely around it, and thus again suggests some independent motion. It might equally well be explained, however, as the result of chromatic attraction all along its length. The two pieces of evidence considered together do, however, indicate a possible active movement of the male nucleus, though they are by no means conclusive".

Wulff (1933) attempted to obtain more definite evidence on the question by working with living pollen tubes stained in neutral red. He continued his observations from the time the generative cell entered into the pollen tube, unless it had already divided in the pollen grain, to the formation of the sperm cells and their further progress into the tube. He found that the generative cell as well as the sperm cells, produced from it at a later stage, usually occupy almost the entire width of the tube and that the vegetative cytoplasm shows fine plasma strands running in opposite directions (*cf.* Beck and Joly, 1941). Now, if the transport of the male gametes were to depend on these strands alone, their passage to the ovule would at best be an extremely slow and uncertain affair. The fact, however, that their forward progress is steadily maintained in spite of the two-way movement of the general cytoplasm of the pollen tube, indicated that they had their own power of locomotion. Wulff also noted that it was only during its division to form the two male gametes that the movement of the generative cell becomes slowed down or temporarily arrested. This is significant from another point of view. In an article entitled "Beziehung zwischen Zellteilung und Zelltätigkeit" Peter (1930) points out that mitosis and the general functions of the cell interfere with each other and that a cell in process of division is unable to work: "Mitose und Zellfunktion hemmen einander; eine in Mitose befindliche Zelle arbeitet nicht . . . eine Zelle, die stark arbeitet, teilt sich nicht mitotisch, eine Zelle, die sich mitotisch teilt, arbeitet nicht. Erhöhte Tätigkeit verhindert die indirekte Teilung. Muss sich eine Zelle doch mitotisch vermehren, so unterbleibt sie ihre Arbeit". Now, if the generative cell were to be carried passively by the streaming movements of the cytoplasm of the pollen tube, its forward movement should continue whether it is in the resting stage or in the process of division to form the two male cells. If, on the other hand, it has its own power of locomotion, we would expect it to cease moving during the period for which it is engaged in the relatively complicated process of mitosis. Since the latter is actually the case (see Wulff, 1933; Fuchs, 1936) and the generative cell lies quiescent during the metaphase and early anaphase and resumes its movement only in late anaphase or telophase, it is reasonable to suppose that it has its own power of locomotion, and the same would then be true of the sperm cells formed by its division. Further observations on living pollen tubes are, of course, desirable, not only to confirm or reject this view but also to see what other factors may operate to bring about or assist this movement[29].

More difficult still is the question whether the male gametes also retain their motility after their discharge into the embryo sac, for direct observations on the latter are almost impossible. Nawaschin's

[29] According to Beck and Joly (1941), it is the turgor of the pollen tube which causes the movement of the generative cell towards its tip.

positive statements on the point are based entirely on their twisted and "worm-like" appearance: "Nach der Gestalt dieser Kerne zu urteilen, scheint es mir ferner gestattet, den Schluss zu ziehen, dass die männlichen Kerne, während sich dieselben noch frei im Protoplasma des Embryosackes befinden, die Fähigkeit zu selbständiger Bewegung besitzen, welche letztere mit der Bewegung eines sich windenden Wurmes vergleichen lässt". There are, however, other plants in which the male gametes are round or oval and the shape gives no suggestion of motility, and in a recent work on *Epipactis latifolia* Hagerup (1945) writes that "the conveyance of the male nuclei probably takes place by means of currents in the cytoplasm which is extended in long strands between the various parts of the embryo sac".

It appears, therefore, that there is no direct evidence to indicate that the male gametes retain their motility after their discharge into the embryo sac. On *a priori* considerations one would expect, nevertheless, that if they have such power while inside the pollen tube, they would continue to preserve it. Further, since nuclei are known to move and change their positions in other plant parts, there is no reason why the male gametes which have only a thin sheath of cytoplasm around them should not be able to act likewise.

THE VEGETATIVE NUCLEUS

In contrast to the generative nucleus, the vegetative, or tube, nucleus stains rather feebly and often presents an abnormal appearance which is indicative of a state of degeneration or decay. In the cleistogamous flowers of *Viola* (Madge, 1930) it becomes four times as long as broad, and in *Vallisneria* (Wylie, 1923) 27 times. In some members of the Labiatae also the elongation is sufficiently pronounced to give it a filamentous outline (Finn, 1939). In *Lilium regale,* according to O'Mara (1933), it presents a variable appearance and seems to be capable of changing its form according to the forces acting upon it in the pollen tube. In certain Chenopodiaceae belonging to the genera *Chenopodium, Atriplex* and *Salsola* (G. O. Cooper, 1935), and in *Lilium candidum* (Herrig, 1922), it has been reported to disintegrate and diffuse into the surrounding cytoplasm of the pollen grain or pollen tube.

Wulff (1933) tried to show that cases of a supposed degeneration or disappearance of the tube nucleus, as had been reported by

Shattuck (1905), Dahlgren (1916), Rocén (1927) and Poddubnaja-Arnoldi (1933), are really to be explained by its lack of affinity for the commonly used stains. By using the Feulgen method he was able to demonstrate it in several plants where it was otherwise on the verge of invisibility. Poddubnaja-Arnoldi (1936) has, however, restated her position and brought forward the following arguments to show that the tube nucleus has no important function in the further development of the pollen tube:

(*a*) It often enters the pollen tube only after the latter has attained an appreciable length, and in many cases it does not lie at the tip of the tube, where it would be most needed if it had any purpose to serve.

(*b*) Often it does not precede the generative cell, or the male gametes, but lies considerably behind: *Elatine* (Frisendahl, 1927), *Brassica* (Pearson, 1933), *Lilium, Convallaria, Galanthus,* (Trankowsky, 1931), *Acacia* (Newman, 1934), *Cannabis, Nicotiana, Papaver, Pisum, Solanum, Secale, Crepis, Allium, Tradescantia* (Poddubnaja-Arnoldi, 1936). In *Vallisneria,* according to Wylie (1923), "the relative position of the tube nucleus and paired sperms in the pollen tube seems to be a matter of chance. Based on a considerable number of observations it was noted that the tube nucleus follows the sperms about as frequently as it precedes them".

(*c*) Apart from a varying position in the tube, the tube nucleus often begins to show signs of disintegration, even before the pollen grain has begun to germinate. It becomes structureless, strongly "zusammengezogen" and irregular in outline, and may even fragment into smaller bits which are apparently quite functionless: *Eichhornia* (Smith, 1898), *Lilium* (Chamberlain, 1897), *Hemerocallis* (Fullmer, 1899), *Asclepias* (Frye, 1902), *Armeria* (Dahlgren, 1916), *Beta* (Oksijuk, 1927), *Scirpus* (Piech, 1928), *Senecio* (Poddubnaja-Arnoldi, 1933) and *Musa* (Juliano and Alcala, 1933).

(*d*) Occasionally, either because of its early degeneration or for some other reason, it does not even enter the pollen tube which, nevertheless, continues to grow normally: *Ulmus* (Shattuck, 1905), *Senecio, Crepis, Secale* (Poddubnaja-Arnoldi, 1936). In certain other instances, *e.g., Aconitum, Cucurbita* and *Papaver,* where the pollen tubes sometimes become branched, the individual branches continue their growth for an appreciable period, although only one of them contains the vegetative nucleus.

Poddubnaja-Arnoldi (1936), therefore, concluded that "der vegetative Kern scheint aus allen diesen Gründen keine aktive Funktion zu besitzen und ist eher als ein atavistisches Merkmal anzusehen".

A similar view has been expressed by Suita (1936, 1937*a*, *b*) who has studied the contents of the pollen grains of *Crinum* with the help of the Feulgen method. He says that soon after the microspore nucleus has completed its first division, the tube nucleus increases in size, and "its chromatic substance disappears". During the development of the pollen grain it becomes amoeboid and is only faintly stained, indicating a decomposition of its thymus nucleic acid. It is therefore concluded that it is a degenerating element which does not play any important part in the life of the pollen tube.

In a work on *Lobelia amoena,* Hewitt (1939) also supports the same idea and gives a list of plants in which the tube nucleus is known to degenerate either in the pollen grain itself or in early stages of pollen tube development.

While further evidence would be welcome, the old view regarding the importance of the vegetative nucleus in the growth and direction of the pollen tube now seems to be less plausible[80, 81].

THE POLLEN TUBE

Germination of pollen. The pollen grains of many species readily germinate in a sugar solution of appropriate strength or even in water. Martin (1913) successfully germinated the pollen of *Trifolium pratense* on hog's bladder moistened with distilled water and was led to the conclusion that the only use of the stigma lies in controlling the water supply[82]. Katz (1926) agreed with this view and said that the chief function of the stigmatic secretion consists in protecting the pollen as well as the stigma from desiccation and that it does not exercise any significant chemical stimulus in the germination of the pollen. She added that the fatty contents

---

[80] A comparable instance of a cell functioning without its own nucleus is that of the sieve tube.

[81] At present the terms "tube nucleus" and "vegetative nucleus" are considered to be synonymous, but if it is definitely proved that this nucleus has no function in directing the growth of the pollen tube, it might be desirable to drop the former name altogether.

[82] An important factor in the germination of pollen grains is that they must lie in fairly intimate contact with the stigmatic papillae. In such cases where they remain together in tetrads, it is possible that only those which are adjacent to the stigmatic secretion are able to germinate (see Wylie, 1904, on *Elodea*).

of the secretion hinder evaporation and thus help in the retention of the necessary moisture required for germination[38]. Light and moderately high temperatures increase this secretion, which is therefore seen in larger quantities in bright sunny weather than on cool rainy days or at night. Minimizing the rôle of the stigma in the reception and germination of pollen, Katz further said that in her experiments the pollen germinated equally well even on the cut surface of the style if only the stigmatic secretion was brought to the stump and the latter kept moist for some time.

Germination of pollen grains is also known to take place within the stylar canal or on the surface of the ovule. Johri (1936*b*) once noted a row of six pollen grains in the hollow stylar canal of *Butomopsis lanceolata,* and in another carpel belonging to the same flower a group of eight pollen grains was found in a similar position. In one case a pollen grain had germinated in the style and in another on the surface of the ovule.

Pollen grains may also germinate on other parts of the flower besides the pistil. In *Aeginetia indica* (Juliano, 1935), for instance, such germination sometimes takes place on the moist surface of the corolla tube, and in cleistogamous flowers (Frisendahl, 1927; Madge, 1929; West, 1930; Maheshwari and Singh, 1934) within the anther lobes themselves. Also, pollen grains of widely separated species and genera often germinate quite readily on a foreign stigma (Strasburger, 1886; Jost, 1905; Sanz, 1945), and in certain cases this has led to the production of interspecific and intergeneric hybrids which are of considerable interest. In *Phaseolus vulgaris* (Weinstein, 1926) the great majority of pollen tubes do not penetrate the stigma at all but grow along its outer surface.

As a rule, however, the stigma does exercise a considerable degree of specificity as to the kind of pollen which will germinate upon it. Ordinarily, pollinations between widely differing species are unsuccessful, either because the pollen grains do not germinate, or, if germination occurs, because the pollen tubes refuse to grow down the foreign style. But sometimes the reverse is true and the pollen shows a very weak germination or none at all on "own" stigma (*e.g., Prunus cerasus, Dahlia* and *Freesia*). More complex relations also may occur between the stigmas and pollen grains of certain plants. In *Cytisus Laburnum* (Jost, 1907), for instance, if the flower is artificially selfed, the grains do not germinate. But if it is insect-pollinated, in which case only cross-pollination occurs, slight wounds are inflicted on the stigma by the visiting bee which enable the pollen to germinate. The same result can also be brought about by artificial wounding, after which own pollen is also capable of germinating and effecting fertilization.

---

[38] A common method used by breeders to increase the germination of pollen is to fit a closed straw over the stigma. This increases the humidity and at the same time excludes foreign pollen, but only large flowers are suitable for such treatment. In *Spathodea campanulata* and several other plants (see Newcombe, 1924) the stigma lobes close up after pollination has taken place; this is said to promote the germination of pollen and is considered essential for this purpose in dry weather.

Course of the pollen tube. During germination of the pollen grain the intine protrudes through a germ-pore[34] and continues to elongate, making its way down through the stigmatic and stylar tissue into the ovary. Only the distal part of the tube has living cytoplasm, and as the nuclei pass forward, callose plugs are formed in the empty portion behind them[35].

Most pollen grains are monosiphonous, *i.e.,* only a single tube is formed from each pollen grain, but others, like those of the Malvaceae, Cucurbitaceae and Campanulaceae, which have many germ-pores are polysiphonous. In *Althaea rosea* as many as 10 pollen tubes, and in *Malva neglecta* even 14, are known to come out from the same pollen grain (see Stenar, 1925, p. 37). Eventually, however, only one of them makes further progress.

Usually the passage of the pollen tube is inter- and not intracellular, but in *Alopecurus* and *Phleum,* Ziegenspeck (1926) noted certain thin areas in the walls of the stigmatic papillae which he calls "Amyloidfenster" because of their association with a starch deposit. These less resistant patches were found to serve as portals for entry of the pollen tube. In stigmas, on the other hand, which had lost their receptivity, the starch deposit had disappeared and the walls of the "windows" had become more resistant. In *Zea mays* (Miller, 1919) the pollen tube may either penetrate a stigmatic hair or grow along it to its base and then enter the silk.

[34] It is interesting to note that in the pollinia of the Mimosaceae (Newman, 1934) and Orchidaceae (Hagerup, 1944) the pollen tubes emerge towards the inside of the pollinium, while in those of the Asclepiadaceae (Gager, 1902) germination takes place through the outer surface of the pollinium.

[35] Little is known of the structure of the wall of the pollen tube which seems to be capable of considerable expansion. Ishikawa (1918), who studied the apical portion of the tip of the pollen tube in *Oenothera,* says that the wall contains cellulose and pectic substances, and that after discharge of the contents of the tube it becomes lined with some peculiar substances, presumably of a mucilaginous nature.

The style is extremely variable in length. In some plants it is so short that the stigma is commonly described as sessile; in corn, on the other hand, it may have a length of 15–30 cm. or more and is stigmatic in all parts of its surface, although germination occurs more satisfactorily near the tip. Depending on the presence or absence of the transmitting tissue, styles have been classified into three main types (Hanf, 1935) : open, half-closed and closed. In the first type there is a wide stylar canal without any true transmitting tissue, and the inner epidermis itself assumes the function of the nutrition and conduction of the pollen tube, as in the Papaveraceae, Aristolochiaceae, Ericaceae and many monocotyledons. In the second type the canal is surrounded by a rudimentary transmitting tissue composed of two or three layers of glandular cells, as in several members of the Cactaceae and in *Campanula* (Barnes, 1885) and *Juglans* (Nawaschin, 1900). In the closed or solid type there is no open channel, and the pollen tubes pass through a core of elongated and richly protoplasmic cells forming the so-called transmitting tissue. As examples may be mentioned *Datura* (Buchholz, 1931) and *Gossypium* (Iyengar, 1938). Finally, there are some plants (*Salix, Acacia,* and many grasses) in which a well marked transmitting tissue seems to be absent and in which there is no special provision for passage of the pollen tube.

As a rule, therefore, the pollen tube makes its way either on the surface of certain glandular cells (often in the mucilage secreted by them) or through the intercellular spaces between the cells of the transmitting tissue, enlarging them by the hydrostatic pressure of its contents. In addition, it probably secretes some enzymatic substances which bring about a dissolution of the middle lamellae of the cells and thus make the path easier (Paton, 1921). Only rarely does it pass through the cells themselves.

After arriving at the top of the ovary, the tube may enter the ovule either by way of the micropyle or through some other route. The first is the more usual condition and is known as "porogamy", but even in plants ordinarily considered as porogamous the entry may not be effected through the micropyle. To mention a few instances, in *Acacia* (Newman, 1934) the integuments are still below the apex of the nucellus at the time of fertilization; and in *Philadelphus* (Mauritzon, 1933), *Utricularia* (Kausik, 1938), *Vandellia* and *Torenia* (Krishna Iyengar, 1940, 1941) the embryo sac protrudes out of the micropyle so that the pollen tube comes in direct contact with it. In the Loranthaceae and Balanophoraceae there are no integuments and therefore nothing that can properly be called a micropyle. In some genera of the former, *viz.*, *Scurrula* and *Dendrophthoe* (Rauch, 1936; Schaeppi and Steindl, 1942), the embryo sacs undergo a remarkable elongation, meeting the pollen tubes half way up in the style. Finally, there are a few cases on record, where, although an integument (or integuments) is present, its lips come in such intimate contact that for all practical purposes a micropyle is absent: *Ficus carica* (Tischler, 1913), *Cynomorium* (Juel, 1902–1903) and *Gunnera* (Samuels, 1912). In *Boerhaavia* (Maheshwari, 1929) the tube actually enters through the micropyle, but before doing this it makes a horizontal crossing through the funiculus. In *Gossypium* (Gore, 1932) it often passes from the funiculus to the base of the ovule and then travels up along the wall of the latter to enter the micropyle.

Chalazogamy or basigamy, which was reported for the first time in *Casuarina* (Treub, 1891), occurs in several members of the Amentiferae, but is not confined to them, being known also in such widely separated genera as *Rhus* (Grimm, 1912) and *Circeaster* (Junell, 1931). It is interesting to note that even in those cases, where penetration into the ovule is effected through the chalaza, the tube usually continues to grow up over the surface of the embryo sac and enters it only after arriving near the egg apparatus: *Ostrya carpinifolia* (Finn, 1936), *Juglans regia* (Nast, 1941), *Casuarina equisetifolia* (Swamy, 1944).

Mesogamy, where the pollen tube enters the ovule by some other route, such as the tissues of the funiculus or the integuments, is less frequent, but occurs sometimes in *Cucurbita* (Longo, 1901; Kirkwood, 1906), *Alchemilla* (Murbeck, 1901), *Epilobium* (Werner, 1914), *Ostrya* (Finn, 1936) and probably in a few other genera.

Considerable phylogenetic significance was attached in the past to the route by which the pollen tube entered into the ovule. Now, this is considered to be of physiological rather than phylogenetic importance, for we sometimes find considerable variation in this respect, even in the same species. For instance, in *Brassica oleracea,* according to Thompson (1933), the tube normally enters through the micropyle, but sometimes it may do so by way of the chalaza. In *Epilobium* (Werner, 1914; Täckholm, 1915) also, the tube may enter through the micropyle, the chalaza or some intermediate route. In *Blainvillea rhomboidea,* Doll (1927) reports having seen two instances in which the pollen tube followed a lateral path along the integumentary tapetum and finally entered the embryo sac through the elongated nucellar cells at its chalazal end.

Mention may be made here of the "obturator", which is an organ serving to facilitate entry of the pollen tube into the ovule. Usually it arises as a swelling of the placenta which grows towards the micropyle and fits like a hood over the nucellus so as to serve as a sort of bridge for the pollen tube (Dahlgren, 1916; Maheshwari and Johri, 1941). Often the cells resemble those of the endotrophic transmitting tissue of the style, or they may be like hairy processes, or sometimes they may have a glandular appearance. In the Combretaceae (Mauritzon, 1939; Fagerlind, 1941*a*), some of them actually penetrate into the micropyle and come in intimate contact with the apex of the nucellus.

During recent years some other structures have also been described, which serve a similar function and may therefore be included under the same general designation, although their morphological origin is different. In the Thymelaeaceae, for instance, it is the cells belonging to the base of the stylar canal which elongate and grow down towards the nucellus (Fuchs, 1938; Venkateswarlu, 1945). A similar "tuft of papillate cells" extends from the bottom of the stylar canal to the apex of the ovule in *Pilea angulata* (Fagerlind, 1944). In *Leucosyke capitellata* and *Myriocarpa longipes,* on the other hand, the cells of the inner integument elongate upward and penetrate into the stylar canal.

In a few instances, like members of the Cucurbitaceae, Euphorbiaceae, Nyctaginaceae (see Schnarf, 1931) and Malpighiaceae (Stenar, 1937; Subba Rao, 1940, 1941), the apex of the nucellus grows out even with the top of the integument or integuments. In *Polygonum persicaria* (Souèges, 1919) the outgrowth is long enough to project into the stylar canal.

Usually there are no special modifications in the cells lining the micropylar canal, but sometimes, as in *Berkheya* (Gelin, 1936), and *Grevillea* (Brough, 1933), they become glandular and seem to contribute to the nutrition and easy passage of the pollen tube. In *Cardiospermum* (Kadry, 1946) the cells of the inner integument, which line the micropylar canal, as well as those belonging to the apical portion of the nucellus, form a mucilaginous mass which serves the same function.

After the pollen tube has reached the nucellus it usually has to travel only a short distance to enter into the embryo sac[36]. In plants with a many-layered nucellar tissue, *viz.*, *Beta* (Artschwager and Starrett, 1933) and *Jussieua* (Khan, 1942), those cells which lie in a direct line with the micropyle often become narrow, elongated and richly protoplasmic, and seem to be designed to lead the pollen tube through the path of least resistance.

It is of interest to note that even during its passage through the nucellus the pollen tube usually passes between the cells and not through them. Only in some plants, such as the Oenotheraceae and Cucurbitaceae, the pollen tubes are extremely broad and actually seem to bore their way forward, destroying many of the cells which lie in their way and causing a permanent break in the tissues.

Entry of pollen tube into the embryo sac. Although usually narrow during its course through the style and the ovary the tube broadens out slightly on coming in contact with the wall of the embryo sac. After penetrating the latter, it makes its way between the egg and one synergid, as in *Fagopyrum* (Mahony, 1935), *Melilotus* (Cooper, 1933), and *Euchlaena* (Cooper, 1937); or between the embryo sac wall and one of the synergids, as in *Cardiospermum* (Kadry, 1946); or directly into a synergid, as in *Taraxacum* (Warmke, 1943) and *Oxalis* (Krupko, 1944). In *Viola* (Madge, 1929) it not only enters a synergid but "forces its way through, or dissolves away, the wall at the base of the synergid before it discharges its contents".

As a rule, only one synergid is destroyed by the pollen tube and the other remains intact until some time afterwards, as in *Crepis* (Gerassimova, 1933), *Viola* (Madge, 1929; West, 1930), *Beta* (Artschwager and Starrett, 1933), *Allium* (Weber, 1929), *Schilla* (Hoare, 1934), *Drosera* (Trankowsky, 1938) and *Vallisneria* (Wylie, 1941). Sometimes both synergids are destroyed, as in *Myosurus* (Tschernojarow, 1926); and in other cases neither is affected, as in *Galinsoga* (Popham, 1938), *Adoxa* (Lagerberg, 1909), *Fagopyrum* (Mahony, 1935), *Santalum* (Rao, 1942) and *Cardiospermum* (Kadry, 1946). Cooper (1931, 1933, 1935*a*,

---

[36] In many plants belonging to the Sympetalae and to some other families (see Dahlgren, 1927*b*) the nucellus is thin and ephemeral and becomes disorganized at the time of maturity of the embryo sac.

1937, 1938, 1940, 1941) also did not find any immediate breakdown of the synergids in the plants investigated by him, *viz., Lycopersicum, Melilotus, Medicago, Euchlaena, Pisum, Portulaca* and *Phryma;* and in *Euchlaena* he found them intact until five days after fertilization.

In addition, there are, of course, some plants where synergids are never formed, *viz., Plumbago, Vogelia* and *Plumbagella* (see Maheshwari, 1948). In others they degenerate even before the entry of the pollen tube, *viz., Tacca, Wormia* (Paetow, 1931) and *Nelumbo* (Ohga, 1937). This seems to indicate that the synergids are not essential for fertilization, and the view that they secrete substances in the nature of proteids, hexoses or pectins, which exercise a chemotactic influence over the pollen tube, or that they act as shock-absorbers against its impact, does not seem to rest on a sound basis[87].

There is no positive information regarding the exact manner of discharge of the male gametes from the pollen tube. Fagerlind (1939) noted some instances in *Peperomia* in which the tip of the tube had divided into two short branches, one of which was directed towards the egg. Cooper (1940, 1941, 1946) also refers to a bifurcation of the tip of the pollen tube in *Portulaca, Phryma* and *Petunia,* one branch becoming closely appressed to the egg and the other extending in the direction of the polar nuclei, and suggests that the two male gametes reach their respective destinations by way of these separate branches. In *Taraxacum kok-saghys* (Warmke, 1943) the same result is brought about by the tip of the pollen tube becoming "wedged in" between the egg cell and the polar fusion nucleus so that both male gametes are in close proximity to their mates.

Branching, persistence and other abnormal behavior of pollen tube. Normally pollen tubes follow a straight course from the stigma to the ovule, but in some plants they are known to branch and ramify like a fungus mycelium. Such a tendency is especially frequent in the Amentiferae and has been described in detail in *Fagus sylvatica* (Finn, 1928*b*). In *Ulmus,* Shattuck (1905) speaks of their "branching and apparently aimless wandering through the funiculus, integuments, and occasionally the nucellus". Branching of a lesser order is known in plants of other groups also. Thus, in *Iris* (Sawyer, 1925) it is common for the tube to fork on reaching the nucellus, and occasionally it ends in three branches. In *Impatiens fulva,* according to Caroll (1919), one branch of the pollen tube may contain one male gamete and the other the second male gamete. In *Myosurus minimus* (Tschernojarow, 1926) the tubes frequently branch in the ovary as well as in the micropyle, and in *Carpinus betulus* (Benson, Sanday and Berridge, 1906) one instance has been reported in which it seemed that the egg cells of two different embryo sacs had been fertilized by branches of the same pollen tube.

---

[87] In his studies on fertilization in *Fritillaria pudica,* Sax (1916) also writes: "There is no evidence to show that the synergid empties its contents into the micropyle in order to aid in the entrance of the pollen tube, as has been suggested by several writers. The fact that all the cells of the egg apparatus are intact at the time the tip of the pollen tube is in contact with the wall of the embryo sac excludes such possibilities in *Fritillaria pudica*".

As a rule, the pollen tube collapses soon after the male gametes have been released, and there is little evidence of it during embryonal development. There are a few cases on record, however, where it is known to persist for longer periods. In *Galinsoga ciliata* (Popham, 1938) it was recognizable up to the seven-celled stage of the embryo, and in *Ulmus americana* (Shattuck, 1905) up to the 16–20 cell stage. In *Hicoria pecan,* according to Woodroof (1928), it persists for two to three weeks or even longer, and the dead end of a tube was once seen beside the fertilized egg, even seven weeks after pollination. In *Elodea, Vallisneria* (Wylie, 1904, 1923) and *Jussieua* (Khan, 1942) the enlarged tips of pollen tubes were found to remain turgid and clearly recognizable until the embryo was "well developed".

In the anomalous embryo sacs of *Isomeris arborea* (Billings, 1937), which are said to consist of only two synergids and an "endosperm" nucleus, the tip of the pollen tube is reported to enlarge and form a "globose terminus", which develops a thick wall and persists until the endosperm is "well along in its development". The contents of the pollen tube are not released, and the embryo is said to arise apomictically "by a direct outgrowth of an endosperm nodule"[38].

Cook (1909) noted a very peculiar behavior of the pollen tube in some material of *Passiflora adenophylla* collected from the botanical garden at Santiago de las Vegas in Cuba. Although normal double fertilization was observed several times, in the majority of cases the pollen tube did not discharge its contents but continued its growth within the embryo sac, eventually filling it and becoming greatly twisted and tangled in the process. Sometimes its growth was so vigorous that all the contents of the embryo sac, including the egg apparatus, were completely absorbed. A few years later (1924) he noted a similar instance in an ovule of *Crotalaria sagittalis* where the pollen tube had penetrated the embryo sac and destroyed its contents. Cook considers these to be examples of an extreme parasitization of the male gametophyte on the female.

---

[38] This account needs confirmation with regard to the development of both the embryo sac and the embryo (see Maheshwari, 1946, 1947; Tischler, 1942/43).

More interesting still is the possibility that in certain cases the pollen tube may serve as an haustorial organ, not for its own benefit but for that of the embryo sac or embryo. Longo (1903) reported such an instance in *Cucurbita.* He found that, owing to a cutinization of the walls of the nucellar epidermis coupled with the formation of a suberized hypostase in the chalazal part of the ovule, the embryo sac of *Cucurbita* becomes cut off from the usual sources of its food supplies. The pollen tube is said to compensate for this deficiency, however. Upon approaching the tip of the embryo sac, it expands into a bladdery swelling or bulla which is often larger in diameter than the embryo sac itself. The bulla gives out a number of branches, one of which penetrates into the embryo sac and effects double fertilization, but the others ramify into the tissues of the nucellus and inner integument and eventually extend along the inner layers of the outer integument, absorbing food materials from the adjacent cells and conducting them to the embryo. It is stated that in those ovules where an embryo was not formed, the haustorial branchings of the pollen tube were also absent.

A somewhat similar phenomenon has been reported in certain members of the Oenotheraceae (Werner, 1914; Täckholm, 1915), in which the pollen tube often broadens considerably in the micropyle and sends out branches into the outer integument and nucellus, while the tip continues to grow toward the embryo sac, destroying the cells in its path[39]. Even after fertilization has taken place, the tube remains recognizable until the embryo is appreciably large. In *Epilobium angustifolium,* in particular, Werner (1914) expressed the opinion that the pollen tube served to correct the deficiency caused by the absence of the antipodal cells[40], so that although the embryo sac itself had a greatly reduced organization it was now enabled to have an additional source of food supply from its upper end.

---

[39] A broad and persistent pollen tube of this kind may sometimes be mistaken for a synergid haustorium or a micropylar extension of the embryo sac. Karsten (1891), for instance, reported that in *Sonneratia apetala,* after fertilization was over, the embryo sac penetrated through the overlying layers of the nucellus and came to lie in direct contact with the nucellar epidermis. The reinvestigations of Venkateswarlu (1937) and Mauritzon (1939) have shown, however, that this is incorrect and that Karsten was actually looking at a part of the pollen tube.

During recent years another report of a similar nature has been made in *Carica papaya,* in which, according to Foster (1943), the pollen tube is a large and massive structure persisting up to at least 64 days after pollination. Owing to its close association with the embryo, the author considers it probable that it may be of assistance in absorbing nutritive substances for the embryo.

Number of pollen tubes entering an embryo sac. Usually only one pollen tube enters an ovule. In a *Lychnis* hybrid Compton (1912) described one ovule with two embryo sacs, each of which had been penetrated by a separate pollen tube. Another, with a single embryo sac, also showed two pollen tubes, but in this case only one of them had actually entered the sac, "the other stopping short half-way through the peculiar specialized channel which leads from the apex of the nucellus towards the embryo-sac". He, therefore, concluded that there is probably "a quantitative relation between embryo-sac and pollen-tube in the matter of chemotaxis, two embryo-sacs secreting enough of the chemotropic substance to attract two pollen-tubes", while one embryo sac could attract only one tube.

Něméc (1931) called attention to certain mechanical contrivances which help to exclude other pollen tubes from entering the ovule after the first has done so. He noted that in *Gagea lutea* the micropyle is at first in close proximity to the glandular tissue of the placenta, but that after the entry of a pollen tube there is a slight elongation of the funiculus causing the ovule to retract from its original position and thereby make it difficult for other pollen tubes to gain entrance.

Apart from the position effect noted by Něméc, there are probably other and hitherto undiscovered factors which also bring about a similar result. In *Scurrula atropurpurea,* Rauch (1936) frequently saw several pollen tubes attached to the wall of the embryo sac, but only one of them entered it, after which the wall of the embryo sac seemed to become firmer and more resistant so as to exclude the remaining tubes. In *Pisum sativum* (Cooper, 1938) also, two or more pollen tubes were frequently seen at the entrance to the micropyle, but only one actually entered it. Recently Pope (1946) has reported that in barley two male nuclei were found within an embryo sac "and six additional ones near the micropyle in four different pollen tubes", but adds that "exactly how the embryo sac admits but one pollen tube has not been determined". In *Phaseolus vulgaris* (Weinstein, 1926) there are many pollen tubes in the style, but only one tube enters each ovule, and the remaining grow on to the basal end of the ovarian cavity where they eventually disintegrate.

---

[40] The embryo sacs of the Oenotheraceae are only four-nucleate, the antipodal cells and lower polar nucleus being absent (see Maheshwari, 1947).

While one pollen tube to an ovule may, therefore, be considered as the general condition in angiosperms, there are nevertheless several reports of the occasional penetration of accessory pollen tubes into the embryo sac. For instance, two pollen tubes have been observed in *Elodea canadensis* (Wylie, 1904), *Ulmus americana* (Shattuck, 1905), *Juglans mandshurica* (Langdon, 1934), *Xyris indica* (Weinzieher, 1914), *Oenothera Lamarckiana* (Ishikawa, 1918), *Vallisneria americana*[41] (Wylie, 1923), *Myosurus minimus* (Tschernojarow, 1926), *Boerhaavia diffusa* (Maheshwari, 1929), *Fagopyrum esculentum* (Mahony, 1935), *Sagittaria graminea* (Johri, 1936*a*) and *Digera arvensis* (Joshi and Kajale, 1937); three in *Statice bahusiensis* (Dahlgren, 1916), *Gossypium* (Iyengar, 1938), *Hedychium* (Santos, 1940) and *Orchis maculata* (Hagerup, 1944); and as many as five in *Juglans nigra* (Nawaschin and Finn, 1912). In *Myosurus minimus* (Tschernojarow, 1926), *Beta vulgaris* (Artschwager and Starrett, 1933) and *Acacia Baileyana* (Newman, 1934) the entry of two or more pollen tubes is said to be a common occurrence.

The presence of accessory pollen tubes in an embryo sac results in the release of supernumerary male gametes which may bring about the phenomenon known as "polyspermy", either in connection with the embryo or endosperm or both. Or, possibly the egg may be fertilized by a sperm from one tube and the polar nuclei by a sperm from another tube. Sprague (1932) has brought forward some indirect evidence of the possibility of such "heterofertilization" in maize.

---

[41] Wylie called it *Vallisneria spiralis*, but it is now generally recognized as a separate species, *V. americana* (Fernald, 1918).

In exceptional cases the same pollen tube may also carry more than two sperms, and this abnormality may originate either before or after germination of the pollen grain. To mention a few instances, three sperms were sometimes seen in the pollen grains of *Cuscuta epithymum* (Fedortschuk, 1931) and four in *Helosis cayennensis* (Umiker, 1920). Dianowa, Sosnovetz and Steschina (1935) saw some pollen grains of *Parthenium argentatum* and *P. incanum* with two vegetative and four sperm nuclei; and Venkateswarlu (1945) noted one pollen grain in *Thymelaea arvensis* with one vegetative and four sperm nuclei, and another with two vegetative and two sperm nuclei. In the pollen tubes of *Allium rotundum* and *A. zebdanense* (Weber, 1929) four to five nuclei were seen; in *Vincetoxicum nigrum* (Guignard, 1922) and *Galanthus nivalis* (Trankowsky, 1931) one vegetative nucleus and four sperm nuclei have been recorded; and in *Spathoglottis plicata* (Suessenguth, 1923) four, five, and in one case even eight, nuclei were seen. Gerassimova (1933) sometimes saw two, three and even five pairs of sperm nuclei in the embryo sacs of *Crepis capillaris* and believes that they were formed by additional divisions of the original pair of sperm nuclei. In artificial cultures of the pollen tubes of *Polygonatum canaliculatum,* Eigsti (1941) noted one tube with three sperms and a vegetative nucleus, and another with four sperms and two vegetative nuclei. In *Taraxacum kok-saghys* (Warmke, 1943) eight sperm nuclei were seen in one instance and ten in another.

Rate of growth of the pollen tube. Computations of the average rate of pollen tube growth are based on measurements of the style and the time elapsing between the landing of the pollen grains on the stigma and the entry of the pollen tube into the ovule. In *Quercus velutina* Lam. (*Q. coccinea* var. *tinctoria* Gray) (Hofmeister, 1858; Conard, 1900) this may be as long as 13 months. In *Hamamelis virginiana* (Shoemaker, 1905) pollination occurs in November or December, and a little later the tips of many tubes reach the base of the funiculus. But here they "hibernate" for the rest of the winter, and growth is resumed only in the spring. In *Corylus* (Hagerup, 1942) pollination takes place in March or April and fertilization in July. In *Hicoria pecan* (Woodroof and Woodroof, 1927) and *Ostrya carpinifolia* (Finn, 1936), the pollen tube takes five to seven weeks to arrive in the ovule, and in *Garrya* *elliptica* (Hallock, 1930) about 17 days, followed a few days later by fertilization. In *Alnus* (Wolpert, 1910) three or four weeks elapse between pollination and fertilization.

Since such long periods are also found in several gymnosperms, it is tempting to infer that this is a primitive feature, but long intervals are also known in the Orchidaceae in which the ovules themselves are formed only after pollination has occurred[42,43]. To mention a few instances, in *Paphiopedilum Maudiae* (a hybrid between *P. callosum* var. *sanderae* and *P. Lawrenceanum* var. *hyeanum*) (Duncan and Curtis, 1942*b*) approximately 19 to 20 weeks elapse between pollination and fertilization; in *P. villosum* (Duncan and Curtis, 1942*b*) it is 14 weeks; in *Phalaenopsis pamala* (Duncan and Curtis, 1942*a*) and *Dendrobium annosum* (Pastrana and Santos, 1930) it is about ten weeks; in *Cattleya* spp. (Duncan and Curtis, 1943) it is about six weeks; in *Cypripedium parviflorum* (Carlson, 1940) it is 26 to 33 days; in *C. pubescens* (Duncan and Curtis, 1942*b*) it is about four weeks; and in *Orchis maculatus* (Hagerup, 1944) about two weeks.

In most plants the period ranges from 12 to 48 hours. Still shorter intervals are not unknown, however. For instance, in *Sorghum vulgare* (Stephens and Quinby, 1934) and *Oryza sativa* (Nagouchi, 1929) it is eight to ten hours; in *Lactuca muralis* (Dahlgren, 1927) six to seven hours elapse from hand pollination to fertilization; in *L. sativa* (Jones, 1927) sperm nuclei were seen in the embryo sac within three hours after pollination, and two hours later fertilization had already occurred in most of the embryo sacs; in *Beta* (Oksijuk, 1934) and *Portulaca oleracea* (Cooper, 1940) the total period is three to four hours; in *Impatiens sultani* (Lebon, 1929) pollen tubes arrive inside the embryo sac in less than an hour after pollination; and in *Parthenium argentatum* (Dianowa *et al.*, 1935), *Crepis capillaris* (Gerassimova, 1933) and *Hordeum distichon* (Persidsky, 1940) fertilization is completed within 60 minutes after pollination. The shortest period on record is in *Taraxacum kok-saghys* (Poddubnaja-Arnoldi and Dianowa, 1934; Warmke, 1943), where fertilization occurs within 15 to 45 minutes after pollination.

---

[42] In *Colchicum autumnale* also, according to an old report of Hofmeister (1858), pollination occurs before the ovules are properly formed, and the embryo begins to develop only six months later.

[43] It must be noted here that not all orchids have a long interval elapsing between pollination and fertilization. In *Epipactis latifolia* (Hagerup, 1945) fertilization occurs within a couple of days after pollination.

Pope (1937) has recently given some data on *Hordeum distichon palmella* for the whole series of events beginning with the germination of the pollen to the end of fertilization. Under greenhouse conditions the pollen grains germinated within eight minutes after reaching the stigma, at ten minutes the male gametes entered the pollen tube, at 40 minutes they had reached the level of the micropyle, at 45 minutes fertilization had begun, at five hours both syngamy and triple fusion had been completed, at six hours the primary endosperm nucleus was in division, and at 15 hours the zygote had also divided.

Usually closely related species show a similar time interval between pollination and fertilization, but such is not always the case. In the "Satsuma orange", for instance, fertilization occurs in about three hours after pollination (Coit, 1914), but in *Citrus trifoliata* a corresponding period of four weeks has been reported (Osawa, 1912).

Several calculations have been made of the average hourly distance travelled by the pollen tube. The following records indicate the range in a few common plants: 1.75 mm. in Pima cotton (Kearney, 1923); 2.1 mm. in *Lilium spp.* (Tokugawa, 1914); 3 mm. in *Datura stramomium* at 23° C. (Buchholz and Blakeslee, 1927); 4 mm. in *Iris versicolor* (Sawyer, 1917); 6.25 mm. in *Zea mays* (Miller, 1919); 15 mm. in *Crepis capillaris* (Gerassimova, 1933); and 35 mm. in *Taraxacum kok-saghys* (Poddubnaja-Arnoldi and Dianowa, 1934). Possibly the actual speed of growth of the tube is still higher, for its path from the stigma to the ovule is not always in a straight line and there are frequent twists and convolutions.

All these records are subject to one important qualification, however. They are true only for the specific conditions under which the observations were made, for it is now well known that the rate of growth of the pollen tube is appreciably affected by environmental conditions. Of these, temperature is the most important. Hofmeister, as early as 1861, observed that in *Crocus vernus,* under conditions of warm moist air and bright sunshine during the day, the pollen tube reached the micropyle in 24 hours, whereas under cooler and drier conditions it took twice or thrice the time. Shibata (1902) reported that in *Monotropa uniflora,* in flowers pollinated on May 3rd, the pollen tubes took 10 days to reach the embryo sac, whereas on June 1st only six days were necessary. He further found that tube growth was adversely affected by low (8°–10° C.) as well as high (above 31° C.) temperatures and that he was thus able to lengthen or shorten the interval between pollination and fertilization at will by altering the environmental conditions. In *Prunus domestica,* according to Dorsey (1919), a low temperature (40°–51° F.) rendered fertilization uncertain because of a retardation of pollen tube growth.

The first detailed and direct study of the question was made by Buchholz and Blakeslee (1927) who found that in *Datura stramonium* the rate of pollen tube growth increased in almost a straight line from a temperature of 52° F. through 57°, 64°, 77° and 84°, reaching a maximum at 92° and dropping down slightly at 98.5° F. It was noted that at 92° F. the growth rate was fully four and a half times that at 52° F.

Since then a somewhat similar range has been reported in some other plants. In tomato (Smith and Cochran, 1935) the maximum growth rate occurred at 70° F., gradually declining at lower as well as higher temperatures. At 100° F. germination was extremely poor; 84 hours after pollination only 3.9 per cent of the pollen grains had germinated and all the tubes were less than 2 mm. long. Even these became abnormally swollen and bulbous at the tips, rendering fertilization improbable. In *Medicago sativa* (Sexsmith and Payer, 1943) no germination took place at 50° F., but there was a steady increase in the rate of pollen tube growth as the temperature was raised from 75° F. to 100° F. In *Hordeum vulgare* var. *pallidum* (Pope, 1943), also, 80° F. was found to be the optimum temperature for pollen tube growth. At this temperature the male gametes reached the embryo sac in about 20 minutes after pollination, while at 41° F. 140 minutes were required for attainment of the same stage. In *Oryza sativa* (Nagouchi, 1931) the optimum temperature for germination of pollen and for tube growth is 30° C., temperatures above 60° C. and below 10° C. being definitely injurious.

It may be concluded that, according to present records (based mostly on observations in temperate regions), pollen germination and tube growth are definitely inhibited at temperatures below 40° F. but occur freely above 50° F. and reach an optimum at 75° to 85° F.[44]

---

[44] Temperature extremes can also affect the development of the plant at

A second factor which markedly affects the rate of pollen tube growth is the degree of compatibility between the male gametophyte and the sporophytic tissues of the pistil. When their reciprocal relations are correct, the pollen tubes travel the full length of the style and fertilization is accomplished before the formation of an abscission layer at the base of the flower. In incompatible matings, on the other hand, the tubes grow very slowly, if at all, and the flower withers before they reach the embryo sac.

The physiological basis for this phenomenon is not clearly understood, for incompatibility reactions may occur not only between different species but within the same species or variety. In *Brassica pekinensis* (Stout, 1931), for instance, the latter condition prevails and may be expressed in the following ways: low percentage of germination of the pollen on "own" stigmas, coiling of the pollen tubes on the stigmatic papillae, feeble or limited growth of the tubes through the style, and coiling of the ends of the tubes in the ovary or ovules. In cross-pollinated flowers of *Petunia violacea* (Yasuda, 1929, 1930), the pollen tubes were found to grow rapidly and show an accelerated rate, reaching the base of the pistil in 36 hours after pollination. In self-pollinated flowers, on the other hand, not only was the growth rate much lower but it also continued to decrease, and the tubes reached only about one-fifth of the length of the style, forming irregular swellings at their tips without being able to effect fertilization. In sugar solutions also, to which an extract of "own" stigma had been added, growth of the tubes was extremely slow, while on addition of the extract from a different strain of the species the tubes grew normally. In *Tradescantia* (Anderson and Sax, 1934) the difference between compatible and incompatible crosses became apparent within 15 minutes after pollination and was increasingly conspicuous thereafter. Although the pollen germinated almost immediately in both cases, growth of the incompatible pollen tubes was much slower and the generative nucleus did not leave the pollen grain, even after 24 hours, while in compatible matings only 40 minutes were required for this process.

---

stages previous to maturation of the gametophytes. In northern Japan, for instance, where the rice harvest is often appreciably lowered due to spells of low temperatures, it has been found that the trouble is caused by a failure of cytokinesis during micro- and megasporogenesis followed by widespread degenerations in both cases (Sakai, 1939; see also Gustafsson and Nygren, 1946).

In *Linaria reticulata* (Sears, 1937) compatible tubes reached the base of the style in less than 25 hours, while incompatible tubes grew only about one-fourth of the distance down the style even in four days' time. In *Nemesia strumosa* (Sears, 1937) incompatible tubes grew approximately as fast as compatible ones through the first three-fourths or four-fifths of the style, but later slowed down rather suddenly and finally came to a stop at the top of the ovary. In a study of incompatible crosses in *Trifolium repens* (Atwood, 1941) two interference zones were observed, one on the stigma and the other in the style. There was poor germination of the pollen, and such pollen tubes as were formed seldom went beyond three-fourths of the way down the style. A few which did proceed further grew so slowly that the flowers withered before any fertilization could take place.

Jost (1907) put forward the view that incompatibility was due to the lack of some essential growth factor operating at various distances in the style. Correns (1912) and East and Park (1918), on the other hand, suggested that the adverse effect was due to the action of certain inhibiting agents ("Hemmungsstoffe") resembling an immunity reaction, whereby the tubes secreted antigens which stimulated the styles to produce antibodies.

An effect, more or less similar to the above, is seen in cases of the so-called "illegitimate pollination" between heterostyled dimorphic and trimorphic plants. In *Fagopyrum esculentum* (Stevens, 1912), for instance, under conditions of legitimate pollination, fertilization takes place in 18 hours, while in illegitimate pollinations more than 72 hours are necessary for the same series of events, and frequently fertilization fails altogether. For further information on this subject see Ernst (1936).

PARTICIPATION OF MALE CYTOPLASM IN SYNGAMY

A question of considerable interest to the geneticist is whether the cytoplasm of the male gamete, as well as its nucleus, enters the egg cell in fertilization. The uncertainty in this respect arises from the difficulty in following the course of events in the embryo sac at this time. One or both of the synergids are demolished, and their cytoplasm together with the cytoplasm of the discharged pollen tube forms a densely staining mass which obscures detail. Added to this is, of course, the further difficulty that the actual process of fertilization takes place very quickly, and that it is only occasionally that one succeeds in "catching" the right stages in the fusion of the gametes.

Wylie (1923), who made a detailed study of the male gametes in *Vallisneria,* said: "It seems certain, however, that some or all of the sperm cytoplasm would enter the egg with the male nucleus". Finn (1925) hinted at the same probability in *Asclepias.* In *Viola riviniana,* according to West (1930), the pear-shaped sperm cells have a small densely staining "dot" in their tailed ends, and, although the cytoplasmic sheath was no longer distinguishable after the sperms had been discharged into the embryo sac, the dot was still visible close to the male nucleus fusing with the egg cell. From this the author concludes that "the first male nucleus is accompanied by some of the cytoplasm, and there is also the probability that this takes part in the fusion". Anderson (1936) likewise, working on *Antirrhinum majus,* tried to identify the cytoplasmic sheath around the male gametes by its extranuclear inclusions and, although his account does not seem to be entirely free from ambiguities, the following quotation seems to indicate the possibility of an inclusion of the male cytoplasm: "The male nucleus which fuses with the polar nuclei is surrounded by mitochondria and the resulting endosperm consequently contains cytoplasmic inclusions from the male plant. The second male nucleus, surrounded by mitochondria, enters the egg and fuses with the egg nucleus. The membrane surrounding the egg, after it has been punctured by the male nucleus, contains an opening large enough for the mitochondria to pass from the pollen tube"[45]. Johri (1936*a, b*), in *Sagittaria graminea* and *Butomopsis lanceolata,* and Smith (1942), in *Camassia leichtlinii,* also traced the male gametes as distinct cells up to the time of their discharge into the embryo sac, but were unable to follow the subsequent events in sufficient detail. The most important contribution on the subject is a recent paper by Wylie (1941), in which he seeks to amplify and extend his previous observations on *Vallisneria*[46].

[45] A doubtful point is whether the mitochondria which entered the egg belonged to the cytoplasm of the male gamete or the general cytoplasm of the pollen tube which is of vegetative origin.

[46] Wylie used a special technique to prepare his material. If fixation was carried out in the ordinary way, the heavy slime inside the ovary impeded the penetration of the killing fluid to such an extent that all the ovules in which pollen tubes had entered got a chance to complete fertilization and the sections did not show the earlier stages in the process. To retard growth, therefore, he cooled the material as soon as collected and squeezed out the

The sperms were found to maintain their identity as cells until they emerged from the pollen tube and arrived in the embryo sac. The initial stages in syngamy were unfortunately lacking, but the repeatedly observed instances of physical contact between the gametic cells suggested their union as protoplasts and not as mere nuclei. Further, the absence of any residue of the first male cell on the surface of the zygote (such as would be expected if only the naked male nucleus had entered the egg), when the same embryo sac plainly showed the cytoplasmic investments left behind by the second sperm, supports the view that the sperm, which fuses with the egg, functions as a cell, and the other entering into triple fusion as a naked nucleus.

Contrary to the above, some other investigators have categorically denied any participation of the male cytoplasm in fertilization. Taking into consideration only the recent literature, Gurgenova (1928) mentions having studied almost all stages of fertilization in *Phelipaea ramosa* C. A. Ney (= *Orobanche ramosa* L.) but observed only sperm nuclei. Madge (1929) saw male cells in the pollen tubes of *Viola odorata* but thinks that the sheath is lost at the time of syngamy. Hoare (1934) reports that in *Scilla nonscripta* the male gametes are "apparently completely naked at the time of fusion". Gerassimova (1933) saw only male nuclei in *Crepis*. Breslavetz (1930) studied *Melandrium album*, using mitochondrial fixatives, but states that her sections of the embryo sac failed to reveal "even the thinnest plasma layer" with chondriosomes or microsomes around the sperm nuclei. Trankowsky (1938) and Gershoy (1940) report the gradual disappearance of the male cytoplasm in their studies of pollen tubes of *Drosera* and *Viola*. Cooper (1946) in a recent paper on fertilization in *Petunia*, says that the X-bodies[47] seen by him in discharged pollen tubes were probably

---

ovules into a beaker of cold water. This slowly dissolved the matrix, and, after repeated decantation and addition of fresh cold water, carried out for several hours, the material was fixed in Flemming's stronger solution. A light Flemming's triple stain was used for staining the sections.

[47] The term "X-bodies" was coined by Nawaschin (1909, 1910) to denote certain dark-staining structures in the tip of the pollen tube or close to it at the time of fertilization. Their exact nature is not understood, and probably it differs in different cases. They have been interpreted as the nuclei of the disintegrated synergids, *e.g.*, *Adoxa* (Lagerberg, 1909); as fragments of the vegetative nucleus, *e.g.*, *Crepis* (Gerassimova, 1933); as supernumerary male nuclei which are functionless and in the process of disintegration, *e.g.*, *Beta* (Artschwager and Starrett, 1933); or even as the nuclei of the adjacent nucellar cells believed to have become shoved into the embryo sac by the impact of the pollen tube, *e.g.*, *Myosurus* (Tschernojarow, 1926).

the remains of the cytoplasmic sheaths of the male gametes, meaning thereby that only the naked nuclei took part in the fusions. In *Tulipa* also, according to Botschanzeva (1937), the male nuclei are said to slip out of their sheaths at the time of fertilization.

It is difficult to dismiss this whole set of observations as based on inadequate technique or imperfect observation. Nevertheless, Finn and Rudenko (1930), in contradistinction to Gurgenova (1928), were able to see the cytoplasmic sheaths around the male nuclei of *Orobanche,* although they could not follow them up to the time of fertilization. Breslavetz's interpretations are open to the objection that plastids and mitochondria may not always be present in the cytoplasm of the male gametes, and her inability to find them is no proof of the absence of the plasma layer itself; in fact, the lighter areas seen by her around the male nuclei are a fairly good indication of the presence of the male cytoplasm. Gershoy's observations, of which only a preliminary account has so far appeared, seem to be contradicted by those of West (1930). In *Crepis* also, although Gerassimova (1933) denies any trace of cytoplasm or of a membrane around the male nuclei, she notes that they are "always" surrounded by a more or less apparent bright "nimbus", which may well be interpreted as the thin hyaline sheath of cytoplasm.

In conclusion, it is necessary to emphasize that until a few years ago the male gametes of angiosperms were as a rule considered to be naked nuclei. The recent work on the subject, more especially from Finn's laboratory (see Finn, 1935, 1940, 1941; Benetskaia, 1939; Kostriukova, 1939*a, b,* 1941, 1945; Kostriukova and Benetskaia, 1939), has left no doubt whatever that the cytoplasmic sheath remains intact, at least so long as the male gametes are in the pollen tube. It would not be surprising, therefore, if with some further improvements in technique it may be possible to observe the details of the process more precisely than has been done up to this time. Finn (1935) has suggested that in order to decide the question of the inclusion of the male cytoplasm with certainty the whole series of events should be studied in living material, but this is difficult in most plants, as the embryo sac is enclosed in several opaque coverings which interfere with any direct and detailed observation of it[48]. The only logical method of attack, therefore, is to find some suitable material in which fertilization stages may be found abundantly, the process does not take place too rapidly, and the cells to be observed not only are fairly large but also respond more favorably to our staining methods. In addition, an attempt should be made, of course, to develop new methods of fixing and staining which will be better adapted to a study of the contents of the embryo sac at the time of fertilization.

---

[48] In a few genera, *e.g., Torenia,* the embryo sac protrudes out of the micropyle, and it may be possible to make direct observations on living material. In certain others, *e.g., Monotropa* and *Orchis,* the seed-coat is thin and transparent. No recent studies have, however, been made on the process of fertilization in any of these plants.

EMBRYO SAC-LIKE POLLEN GRAINS

Němec in 1898 noted that in the petaloid anthers of *Hyacinthus orientalis* the microspores sometimes formed large eight-nucleate structures, remarkably similar to embryo sacs. He believed that they arose as the result of a degeneration of the generative nucleus, followed by three divisions of the vegetative nucleus.

Later De Mol (1923) observed this so-called "Němec-Phenomenon" in the anthers of several varieties of *Hyacinthus orientalis* which had been subjected to certain special conditions by breeders: (*a*) digging up of immature bulbs in June; (*b*) ripening them by treatment with high temperatures (22°–25° C.) at frequent intervals in July and August; (*c*) replanting in September; and (*d*) transferring them in January to a greenhouse to force flowering. He attributed the origin of this abnormality to a duplication of the generative nuclei caused by certain physiological stimuli.

Stow (1930, 1934) obtained similar embryo sac-like pollen grains or "pollen embryo sacs" in the normal anthers of a variety called "La Victoire", obtained from bulbs which had been subjected to a temperature of 20° C. at the time of reduction division and afterwards further forced in the greenhouse. He traced their development more fully than either Němec or De Mol. According to his account, the microspores increase in size to form large sacciform bodies, and the nucleus in each divides to form eight daughter nuclei. Three of these lie at the end where the exine is still attached, three at the opposite end, and two in the middle. The six nuclei at the poles now organize into cells, while the remaining two fuse in the center, thus showing a perfect resemblance to a true embryo sac except for the absence of the enclosing layers of the nucellus and integuments. The three cells at the exine end were found to stain well and remain healthy for a long time, but the other three soon showed signs of degeneration. From this it is inferred that the former are the equivalents of the egg apparatus and the latter of the antipodal cells[49]. In addition, certain abnormal types were also seen: (*a*) with eight nuclei forming an "egg" cell, two "polar" nuclei, and five "antipodal" cells; (*b*) with only four nuclei, forming an "egg" cell, one "antipodal" and two "polar" nuclei, or one "polar" nucleus and three "antipodal" cells without any egg; (*c*) with 16 nuclei, forming a five- to ten-celled "egg apparatus", one or two "polar" nuclei and four or five "antipodals"; and (*d*) with more than 16 nuclei without any definite organization.

According to Stow, it is not the duplication of the vegetative or generative nucleus which produces the pollen embryo sacs but the divisions of the microspore nucleus itself. Once the generative cell has been cut off, further development is entirely normal and no pollen embryo sacs are formed. Also, in his material the latter were always accompanied by a much larger number of dead pollen grains (more than 90% of the total), and he has therefore suggested that the latter produce a "necrohormone" which causes an abnormal development of the surviving ones. He also states that the microspore is potentially capable of developing into either a male or a female organ. Under normal conditions the "male potency" is dominant over the "female potency", so that there is a formation of the generative cell and the male gametes; under abnormal conditions, however, when there is an abundance of the "necrohormone", the "female potency" gets the upper hand, giving rise to embryo sac-like structures.

An interesting phenomenon mentioned in support of this view is that when these pollen embryo sacs were placed on an agar medium together with some normal pollen grains of another variety, the pollen tubes formed from the latter grew towards the former and entwined them. In one case a sperm nucleus was "just getting into the pollen-embryosac", and in another 16 free nuclei were seen, believed to have been derived from the divisions of a triple fusion nucleus. Unfortunately precise evidence of the occurrence of fertilization was not available, but it is possible that the pollen embryo sacs do possess some female tendencies not only in their development and mature organization but also in the physicochemical character of their cytoplasm and consequently in their physiological responses.

---

[49] Recently, in some material of *Ornithogalum nutans* collected from the Botanical Gardens at Vienna, Geitler (1941) found, besides normal and degenerating pollen grains, others where eight and 16 nuclei were present. Those with eight nuclei showed a typical embryo sac organization, but, contrary to Stow, Geitler interprets the three nuclei on the exine side of the pollen grain as antipodals and the three at the opposite end as constituting the egg apparatus.

Shortly after the publication of Stow's papers, Naithani (1937) found embryo sac-like pollen grains in the variety "Yellow Hammer" when the bulbs had been treated by breeders for early flowering. He has confirmed Stow's observations regarding the mode of development of these abnormal pollen grains, but believes their formation to be a temperature effect and not the result of a liberation of "necrohormones" from aborting pollen grains. According to him, the degeneration of the remaining pollen grains is not the cause but the effect of a hypertrophied growth of the more favored ones which use up all the available food for their own growth. On the other hand, De Mol (1933, 1934), working on the varieties "La Victoire" and "Dr. Lieber", suggests that neither the digging up of immature bulbs nor their final exposure to greenhouse conditions are essential factors in the process and that even immature bulbs planted in the open can give rise to embryo sac-like pollen grains, provided they are exposed to a specified heat treatment just at the time of the meiotic divisions in the microspore mother cells, *i.e.*, during the period between July 15th and November 10th. Sax (1935) also, on subjecting the flowers of *Tradescantia* to high and low temperatures at a stage prior to the formation of the microspores, found three kinds of nuclear and cytoplasmic abnormalities: (*a*) a division of the vegetative nucleus, (*b*) a disturbance in the polarity of the cell, (*c*) an unusual extension of the microspores on the ventral side.

It may be concluded that while it is possible to produce embryo sac-like pollen grains in certain plants and under certain environmental conditions, this can so far be considered only as an interesting abnormality, and it is doubtful that these structures can be made to function as true embryo sacs.

SYSTEMATIC VALUE AND HOMOLOGIES OF THE MALE GAMETOPHYTE

In conclusion, some remarks may be offered on the homologies of the male gametophyte. In a speculation of this kind it is natural to turn back to the gymnosperms[50]. Unfortunately we know very little about the structure of the male gametophyte in the extinct orders of the group. The available evidence suggests, however, that the pollen grains were multicellular structures, consisting perhaps of both prothallial and spermatogenous cells. Probably there were no pollen tubes and the sperms swam directly to the archegonia. Ciliated sperms are also present in the living cyads and in *Ginkgo,* but, in addition, a pollen tube is also formed. It is interesting to note, however, that the tube originates from the upper end of the pollen grain and grows into the nucellar tissues, acting as an haustorial and not a sperm-carrying structure. The basal end of the pollen grain hangs free in a cavity which may be said to be composed partly of the pollen chamber and partly of the archegonial chamber. There are present, besides the two sperms, one (in the Cycadales) or two (in the Ginkgoales) vestigial prothallial cells, a stalk cell and a tube nucleus. In one genus, *Microcycas,* there are 16 to 22 sperms, which should probably be considered a primitive feature.

---

[50] For literature citations and more detailed information on this group, see Schnarf (1933, 1941), Chamberlain (1935) and Buchholz (1945).

The Coniferales differ in two important respects: (*a*) the sperms are without any cilia, although it does not necessarily follow that they have entirely lost their power of locomotion; (*b*) the pollen tube, instead of being merely an haustorial structure arising from the upper end of the pollen grain, grows out at the lower end, progressing downward through the nucellus into the archegonium and therefore serving the additional function of being an agent for transporting the male gametes to the egg cell. The contents of the pollen grain and the tube vary in different genera. In many cases there are two prothallial cells, a tube nucleus, a stalk cell and two male gametes, as in *Ginkgo.* In some, like *Araucaria, Podocarpus, Dacrydium* and *Phyllocladus,* there is a considerable prothallial tissue. On the other hand, most of the Taxodiaceae and Cupressaceae, and all the Taxaceae and Cephalotaxaceae, completely lack prothallial cells. Another feature of considerable interest is the great disparity in size between the two sperm cells in *Taxus, Torreya* and *Cephalotaxus,* the smaller cell presumably being on the way to abortion. Some species of *Cupressus* are exceptional in having multiple male cells, all of which seem to be capable of functioning.

Coming to the Gnetales, *Ephedra* with its two prothallial cells, two male gametes, a stalk and a tube nucleus does not seem to differ materially from the preceding gymnosperms like *Ginkgo* and *Pinus*. The inner integument forms a long micropylar canal, but the pollen grains are drawn down the whole distance into the pollen chamber formed by disintegration of the nucellar cells. The pollen tube is short and makes its way through the neck of the archegonium discharging its contents into the egg. *Welwitschia* and *Gnetum* are only imperfectly known, but in both cases the pollen grains seem to have a prothallial cell, a generative cell and a tube cell. The stalk cell is eliminated, and the generative cell directly gives rise to the two male gametes. The pollen tubes may begin to form after the pollen grains have arrived at the tip of the nucellus or sometimes also at some distance behind in the micropylar canal itself. A peculiar feature, known only in *Welwitschia,* is the formation of the so-called "prothallial tubes" from the female gametophyte. These grow about half-way up through the nucellus to meet the downcoming pollen tubes, and fertilization occurs somewhere in the middle.

Summing up then, it seems that although in several gymnosperms a prothallial tissue is present, there has been a tendency towards its elimination, and in many cases the male gametophyte is reduced to a structure consisting of only four nuclei—a tube nucleus, two male gametes and a single prothallial cell. The Cycadales and Ginkgoales still have ciliated sperms, but in the remaining members of the class the cilia have been lost. Associated with this is the evolution of the pollen tube which becomes the channel for transport of the male gametes. In general, the pollen grains alight directly on the nucellus, but in *Larix* and *Pseudotsuga* the lips of the integument become stigmatic on the inner surface, and in *Araucaria* and *Agathis* the pollen may germinate almost anywhere on the ovuliferous scale and the pollen tubes are able to grow up to the ovule. In *Gnetum* germination may frequently take place in the micropylar canal at some distance from the apex of the nucellus.

The male gametophyte of angiosperms may be assumed to have been derived from that of some gymnospermous ancestor like *Gnetum* by further simplification and an elimination of the single prothallial cell[51]. However, this change seems to have been accompanied by the evolution in the angiosperms of the closed carpel and the differentiation of a stigma and style, for which there is no counterpart in the Gnetales. The micropylar canal, which some authors have attempted to homologize with an open style, is a structure formed by the inner integument, and the resemblance is too superficial to enable us to make any profitable speculations in this line. The "prothallial tubes" of *Welwitschia* to some extent resemble the elongated embryo sacs of certain members of the Loranthaceae which reach half-way up into the style, but both of these conditions are too aberrant to be taken into further consideration.

---

[51] There have been occasional reports of the occurrence of a prothallial cell in some angiosperms like *Lilium, Eichhornia, Yucca, Sparganium, Atriplex* and *Stellaria* (see Wulff and Maheshwari, 1938, p. 124), but these are in the nature of freaks, and no great significance can be attached to them. Up to the present there is no record of an angiospermous pollen grain having a prothallial cell as a constant feature.

As with the embryo sac (Maheshwari, 1948) we are also very much in the dark about the origin and evolution of the male gametophyte of angiosperms. The question is so intimately bound up with the origin of the closed carpel and of the stigma and style that it is impossible to arrive at any satisfactory conclusion with the help of the existing data. Possibly a discovery of some new fossil forms may shed some light on this vexed question.

More hopeful, however, is a study of the male gametophyte in the elucidation of the inter-relationships of individual genera and families. Characters of pollen morphology, like the adornments of the exine and the number and position of the germ-pores and furrows, have long been recognized to be of considerable significance in this connection. Schürhoff in 1926 pointed out that the internal structure of the male gametophyte was also of the same importance as the external, and the only reason why it had not been utilized to the same extent was the greater difficulty in obtaining the necessary data of this kind. He considered the number of nuclei in the pollen grains to be the most important character and stated that the two-nucleate condition was the primitive and the three-nucleate the more advanced.

Poddubnaja-Arnoldi (1936) took a different view and sought to minimize the value of this character on the ground that sometimes in the same family, genus or even species both types of pollen grains occur side by side. Further, in certain plants, in which the generative nucleus normally divides in the pollen tube, she was able to induce its division in the pollen grain by altering the environmental conditions.

This objection, although weighty at first sight, is really of little significance, for while it is true that alterations of environment can hasten or delay the division of the generative cell or bring about still other changes, the same is also true of all other characters, whether vegetative, morphological or embryological. As a rule, however, the number of nuclei in the mature pollen grain is a constant feature in every species or genus, and sometimes whole families and orders are characterized by the same type of pollen. To cite a few instances, the pollen grains of the Centrospermales, Umbelliferae, Borraginaceae, Compositae, Helobiales, Gramineae, Cyperaceae and Juncaceae are three-celled almost without exception, while those of the Malvales, Rosaceae, Leguminosae, Bicornes, Scrophulariaceae, Commelinaceae, Pontederiaceae, Amaryllidaceae and Orchidaceae are two-celled. There are, no doubt, other groups like the Rhoeadales, Labiatae, Rutaceae and Rubiaceae, where both two- and three-celled pollen grains are found. There is evidence, however, to indicate that the two types are not irregularly distributed but form sub-groups in a family or order which also show significant differences in other respects.

The number of nuclei is, however, not the only criterion to be taken into consideration. The place where the generative cell is cut off, as well as the shape, size and stainability of this cell, and of the male cells formed from it are also of considerable importance. In the Compositae the male gametes are as a rule greatly elongated; in the Alismaceae they are spindle-shaped; in the Hydrocharitaceae they are often joined in pairs and remain together throughout their passage in the pollen tube. Wunderlich (1936, 1937) has recently used these and other characters of the male gametophyte with considerable skill in her studies of the Liliaceae and Amaryllidaceae, but, as she has herself pointed out, her conclusions need to be checked with the help of evidence from other sources, and they will not therefore be considered further.

A notable instance of the diagnostic value of the male gametophyte is seen in the family Cyperaceae. Here, as is known through the studies of Juel (1900), Stout (1912), Piech (1924*a, b,* 1928), Håkansson (1927) and Tanaka (1939 *a, b,* 1940, 1941), only one of the microspores of a tetrad functions, while the other three fail to grow and are soon crushed and absorbed. In the functioning microspore the first division gives rise to the tube and generative cells after which the generative cell divides again to give rise to the two male cells. This feature is absolutely constant in the family and may be taken as a diagnostic character of it in the same way as a monosporic four-nucleate embryo sac is a characteristic feature of the Oenotheraceae (see Maheshwari, 1947, 1948).

The above peculiarity of the development of the pollen grains in the Cyperaceae is of further interest in speculations concerning its relationships with other families of the monocotyledons. In the Juncaceae also, the four cells of the tetrad remain united into a compound grain, and long ago Wille (1886) said that "die Pollenbildung bei den Cyperaceen sich morphologisch als eine noch mehr reduzierte Entwicklungsform an die der Juncaceen anschliesst und dass sie somit eine Stütze für die Ansicht gibt, dass die Cyperaceen phylogenetisch als reduzierte Juncaceen aufzufassen sind". Wulff (1939*a*) has recently brought further evidence in support of this view, and it seems likely that the Cyperaceae are not related to the Gramineae, as thought by most systematists, but to the Juncaceae.

In conclusion, we may say with Schnarf (1937), who has investigated the pollen grains of numerous species belonging to 134 genera and 60 families, that characters of the male gametophyte, although not superior, are also not inferior to others like the structure of the ovule, the divisions of the microspore mother cells, the formation of the endosperm, the number of perianth lobes and stamens, the position of the ovary, and so on. It is, as he says, fundamentally incorrect to lay undue importance on any single character, for it is only an evaluation of a sum total of *all* characters which can lead to a clear and correct picture of the relationships of a family, genus or species.

ACKNOWLEDGMENTS

For the preparation of this paper, written during the latter part of my tour in the United States, I drew upon the facilities so kindly provided by the botanical laboratories at Smith College, Harvard University, University of Illinois, Cornell University, State University of Iowa, University of Nebraska, University of California at Berkeley and Los Angeles, and Stanford University. I should specially like to mention the names of Prof. J. T. Buchholz, Prof. A. F. Blakeslee, Prof. R. H. Wetmore, Prof. I. W. Bailey, Prof. A. J. Eames, Prof. A. S. Foster, Prof. G. M. Smith and Prof.

A. W. Haupt for their encouragement. To Prof. K. V. O. Dahlgren (Uppsala), Prof. L. E. Anderson (Duke University), Prof. O. J. Eigsti (Northwestern University), Dr. A. V. Beatty (Emory University), Mr. Reayat Khan (Dacca University), and to Mrs. M. S. Cave, Miss Lois Eubank, Miss Florence Signaigo and Miss Jean McEown (University of California) I am indebted for their help in the preparation of the manuscript. I am also thankful to Dr. B. G. L. Swamy (Bangalore) for the preparation of the figure used in this paper for illustrating the typical course of development of the male gametophyte in angiosperms.

LITERATURE CITED

AFIFY, A. 1944. Cytological studies in the Cucurbitaceae and their evolutionary significance. Jour. Genet. **46**: 116–124.

AFZELIUS, K. 1936. Apomixis in der Gattung *Arnica*. Svensk Bot. Tidskr. **30**: 572–579.

ALDEN, ISABEL. 1912. A contribution to the life history of *Uvularia sessilifolia*. Bull. Torrey Bot. Club **39**: 439–446.

ANDERSON, E. and SAX, K. 1934. A cytological study of self-sterility in *Tradescantia*. Bot. Gaz. **95**: 609–621.

ANDERSON, L. E. 1936. Mitochondria in the life cycles of certain higher plants. Am. Jour. Bot. **23**: 490–500.

————. 1939. Cytoplasmic inclusions in the male gametes of *Lilium*. Am. Jour. Bot. **26**: 761–766.

ARTSCHWAGER, E. and STARRET, R. C. 1933. The time factor in fertilization and embryo development in sugar beet. Jour. Agr. Res. **47**: 823–843.

ATWOOD, S. S. 1941. Cytological basis for incompatibility in *Trifolium repens*. Am. Jour. Bot. **28**: 551–557.

BANERJI, I. 1933. The development of the flower and pollen in jute. Indian Jour. Agr. Sci. **3**: 116–126.

————. 1940. A contribution to the morphology and cytology of *Carthamus tinctorius* Linn. Proc. Nat. Inst. Sci. India **6**: 73–86.

———— and GANGULEE, H. C. 1937. Spermatogenesis in *Eichhornia crassipes* Solms. Jour. Indian Bot. Soc. **16**: 289–296.

———— and HALDAR, S. 1942. A contribution to the morphology and cytology of *Monochoria hastaefolia* Presl. Proc. Indian Acad. Sci. B. **16**: 91–106.

———— and SAMAL, K. K. 1936. Microsporogenesis in *Crotalaria juncea* Linn. Indian Jour. Agr. Sci. **6**: 116–126.

BARBER, H. N. 1942. The pollen grain division in the Orchidaceae. Jour. Genet. **43**: 97–103.

BARNES, C. R. 1885. The process of fertilization in *Campanula americana* L. Bot. Gaz. **10**: 349–354.

BEATTY, A. V. 1943. The division of the generative nucleus in *Eschscholtzia*. Am. Jour. Bot. **30**: 378–382.

BECK, W. A. and JOLY, R. A. 1941. Some growth phenomena in cultured pollen tubes. Trans. Am. Micr. Soc. **60**: 149–162.

BEER, R. 1906. On the development of the pollen grain and anther of some Onagraceae. Beih. Bot. Centralbl. **19A**: 286–313.

BENETSKAIA, G. K. 1939. Observations in vivo des gamètes mâles dans les tubes polliniques d'*Asclepias Cornuti* Decsn., de *Vinca major* L. et de *Vinca minor* L. Jour. Bot. URSS **24**: 273–281.

Benson, M. *et al.* 1906. Contribution to the embryology of the Amentiferae. II. *Carpinus betulus.* Trans. Linn. Soc. London. II. Bot. **7**: 37–44.

Billings, F. H. 1934. Male gametophyte of *Atriplex hymenelytra.* Bot. Gaz. **95**: 477–484.

————. 1937. Some new features in the reproductive cytology of angiosperms, illustrated by *Isomeria arborea.* New Phytol. **36**: 301–326.

Blackman, V. H. and Welsford, E. J. 1913. Fertilisation in *Lilium.* Ann. Bot. **27**: 111–114.

Blakeslee, A. F. 1945. Removing some of the barriers to crossability in plants. Proc. Am. Phil. Soc. **89**: 561–574.

Boehm, K. 1931. Embryologische Untersuchungen an Zingiberaceen. Planta **14**: 411–440.

Bose, P. K. 1937. Cytological studies of *Argemone mexicana* Linn. Jour. Indian Bot. Soc. **16**: 197–208.

Botschanzeva, Z. P. 1937. Spermogenesis bei *Tulipa* und seine Erlerung zwecks Kariosystematik. Bull. Univ. Asie Centrale (Taschkent) **22**: 329–338.

Breslavetz, L. 1930. Spermatogenesis and fertilization process in some plants in connection with the question of heredity through plasma. Proc. U.S.S.R. Congr. Genet. **2**: 181–186.

Brink, R. A. and Cooper, D. C. 1936. The mechanism of pollination in alfalfa (*Medicago sativa*). Am. Jour. Bot. **23**: 678–683.

———— and ————. 1938. Partial self-incompatibility in *Medicago sativa.* Proc. Nat. Acad. Sci. **24**: 497–499.

Brough, P. 1933. The life history of *Grevillea robusta* Cunn. Proc. Linn. Soc. New South Wales **58**: 33–73

Brumfield, R. T. 1941. Asymmetrical spindles in the first microspore division of certain angiosperms. Am. Jour. Bot. **28**: 713–722.

Buchholz, J. T. 1931. The dissection, staining and mounting of styles in the study of pollen-tube distribution. Stain Tech. **6**: 13–24.

————. 1945. Gymnosperms. Encyclopaedia Brittanica, 14th ed.

———— and Blakeslee, A. F. 1927. Pollen-tube growth at various temperatures. Am. Jour. Bot. **14**: 358–369.

Capoor, S. P. 1937*a*. Contributions to the morphology of some Indian Liliaceae. II. The gametophytes of *Urginea indica* Kunth. Beih. Bot. Centralbl. **56A**: 156–170.

————. 1937*b*. The life history of *Holoptelea integrifolia* Planch. Beih. Bot. Centralbl. **57A**: 233–249.

Carlson, E. M. and Stuart, B. C. 1936. Development of spores and gametophytes in certain New World species of *Salvia.* New Phytol. **35**: 68–91.

Carlson, M. C. 1940. Formation of the seed of *Cypripedium parviflorum.* Bot. Gaz. **102**: 295–301.

Caroll, F. B. 1919. The development of the chasmogamous and cleistogamous flowers of *Impatiens fulva.* Contr. Bot. Lab. Univ. Pennsylvania **4**: 144–183.

Chamberlain, C. J. 1897. The pollen grain. *In* Coulter, J. M., Chamberlain, C. J. and Schaffner, J. H. Contributions to the life history of *Lilium Philadelphicum.* Bot. Gaz. **23**: 412–452.

————. 1935. Gymnosperms, structure and evolution.

Coit, J. E. 1914. Citriculture. Cal. Agr. Exp. Sta., Rep. **1913/1914**: 105–106.

Compton, R. H. 1912. Note on a case of doubling of embryo sac, pollen tube, and embryo. Ann. Bot. **26**: 243–244.

Conard, A. H. 1900. A contribution to the life history of *Quercus.* Bot. Gaz. **29**: 408–418.

Cook, M. T. 1909. Notes on the embryo-sac of *Passiflora adenophylla.* Bull. Torrey Bot. Club **36**: 373–374.

————. 1924. Development of seed of *Crotalaria sagittalis.* Bot. Gaz. 77: 440–445.

COOPER, D. C. 1931. Macrosporogenesis and the development of the macrogametophyte of *Lycopersicon esculentum.* Am. Jour. Bot. 18: 739–748.

————. 1933. Macrosporogenesis and embryology of *Melilotus.* Bot. Gaz. 95: 143–155.

————. 1935*a*. Macrosporogenesis and embryology of *Medicago.* Jour. Agr. Res. 51: 471–477.

————. 1935*b*. Microsporogenesis and the development of the male gametes in *Portulaca oleracea.* Am. Jour. Bot. 22: 453–459.

————. 1936. Development of the male gametes in *Lilium.* Bot. Gaz. 98: 169–177.

————. 1937. Macrosporogenesis and embryo sac development in *Euchlaena mexicana* and *Zea mays.* Jour. Agr. Res. 55: 539–551.

————. 1938. Embryology of *Pisum sativum.* Bot. Gaz. 100: 123–132.

————. 1940. Macrosporogenesis and embryology of *Portulaca oleracea.* Am. Jour. Bot. 27: 326–330.

————. 1941. Macrosporogenesis and the development of the seed of *Phryma leptostachya.* Am. Jour. Bot. 28: 755–761.

————. 1946. Double fertilization in *Petunia.* Am. Jour. Bot. 33: 54–57.

COOPER, G. O. 1935. Cytological studies in the *Chenopodiaceae.* I. Microsporogenesis and pollen development. Bot. Gaz. 97: 169–178.

CORRENS, C. 1912. Selbsterilität und Individualstoffe. Fests. Math.-Nat. Ges. 84. Versamml. Deut. Naturf. u. Ärzte, Münster (Westf.): 186–217. (Reprinted 1913 in Biol. Zbl. 33: 389–423.)

COULTER, J. M. and CHAMBERLAIN, C. J. 1903. Morphology of angiosperms.

———— and ————. 1910. Morphology of gymnosperms.

DAHLGREN, K. V. O. 1915. Über die Überwinterungsstadien der Pollensäcke und der Samenanlagen bei einigen Angiospermen. Svensk Bot. Tidskr. 9: 1–12.

————. 1916. Zytologische und embryologische Studien über die Reihen Primulales und Plumbaginales. Kung. Svenska Vet.-Akad. Handl. 56(4): pp. 80.

————. 1927*a*. Die Befruchtungserscheinungen der Angiospermen. Hereditas 10: 169–229.

————. 1927*b*. Die Morphologie des Nuzellus mit besonderer Berücksichtigung der deckzellosen Typen. Jahrb. Wiss. Bot. 67: 347–426.

————. 1937. Die Entwicklung des Embryosackes bei *Plumbago zeylanica.* Bot. Not. 1937: 487–498.

DARLINGTON, C. D. 1936. The analysis of chromosome movements. I. *Podophyllum versipella.* Cytologia 7: 242–247.

————. 1937. Recent advances in cytology. 2nd ed.

DARWIN, C. 1894. The variation of animals and plants under domestication. 2nd ed.

DE MOL, W. E. 1923. Duplication of generative nuclei by means of physiological stimuli and its significance. Genetica 5: 225–272.

————. 1933. Die Entstehungsweise anormaler Pollenkörner bei Hyazinthen, Tulpen und Narzisse. Cytologia 5: 31–65.

————. 1934. Näheres über das Vorfinden nebst dem experimentellen Hervorrufen mehrchromosomiger und embryosackartiger Pollenkörner bei diploiden und heteroploiden hollandischer Hyazinthenvarietäten. Cytologia 5: 204–229.

DIANOWA, W. J. *et al.* 1935. Vergleichende zytologische Untersuchung der Varietäten vom *Parthenium argentatum* Gray und *P. incanum* Gray. Beih. Bot. Centralbl. 53A: 293–339.

DIETTERT, R. A. 1938. The morphology of *Artemisia tridentata* Nutt. Lloydia 1: 3–74.

DOAK, C. C. 1937. The pistil anatomy of cotton as related to experimental control of fertilization under varied conditions of pollination. Am. Jour. Bot. **24**: 187–194.

DODDS, K. S. and SIMMONDS, N. W. 1946. Genetical and cytological studies of *Musa*. VIII. The formation of polyploid spores. Jour. Genet. **47**: 223–241.

DOLL, W. 1927. Beiträge zur Kenntnis der Dipsaceen und Dipsaceen-ähnlicher Pflanzen. Bot. Archiv **17**: 107–146.

DORSEY, M. J. 1919. A study of sterility in the plum. Genetics **4**: 417–488.

DUDLEY, M. G. 1937. Morphological and cytological studies of *Calla palustris*. Bot. Gaz. **98**: 556–571.

DUGGAR, B. M. 1900. Studies in the development of the pollen grain in *Symplocarpus foetidus* and *Peltandra undulata*. Bot. Gaz. **29**: 81–98.

DUNCAN, R. E. and CURTIS, J. T. 1942*a*. Intermittent growth of *Phalaenopsis*. A correlation of the growth phases of an orchid fruit with internal development. Bull. Torrey Bot. Club **69**: 167–183.

———— and ————. 1942*b*. Intermittent growth of fruits of *Cypripedium* and *Paphiopedilum*. A correlation of the growth of orchid fruits with their internal development. Bull. Torrey Bot. Club **69**: 353–359.

———— and ————. 1943. Growth of fruits in *Cattleya* and allied genera in the Orchidaceae. Bull. Torrey Bot. Club **70**: 104–119.

DUTT, M. K. 1943. Development of the microspores and the nuclear behavior in tapetal cells of *Putranjiva Roxburghii* Wall. Sci. & Culture **8**: 309–310.

EAST, E. M. 1934. Reaction of stigmatic tissue against pollen-tube growth in selfed self-sterile plants. Proc. Nat. Acad. Sci. **20**: 364–368.

———— and PARK, J. B. 1918. Studies on self-sterility. II. Pollen-tube growth. Genetics **3**: 353–366.

EDMAN, G. 1931. Apomeiosis und Apomixis bei *Atraphaxis frutescens* C. Koch. Acta Horti Bergiani **11**: 13–66.

EIGSTI, O. J. 1939. The morphology of pollen tubes in angiosperms. Proc. Okl. Acad. Sci. **19**: 105–107.

————. 1940. The effects of colchicine upon the division of the generative cell in *Polygonum, Tradescantia* and *Lilium*. Am. Jour. Bot. **27**: 512–524.

————. 1941. The occurrence of a pollen tube with four sperms and two tube nuclei in *Polygonatum*. Proc. Okl. Acad. Sci. **21**: 134–136.

ELFVING, F. 1879. Studien über die Pollenkörner der Angiospermen. Jenaische Zeits. **13**: 1–28.

ERNST, A. 1936. Der Heutige Stand der Heterostylieforschung. Proc. 6th Int. Bot. Cong. Vol. 1: 35–53.

FAGERLIND, F. 1938. Der Embryosack von *Plumbagella* und *Plumbago*. Ark. Bot. **29B**: 1–8.

————. 1939. Die Entwicklung des Embryosackes bei *Peperomia pellucida*. Ark. Bot. **29A**: 1–15.

————. 1941*a*. Der Bau der Samenanlage und des Makrogametophyten bei *Quisqualis indica*. Bot. Not. **1941**: 217–222.

————. 1941*b*. Bau und Entwicklung der *Gnetum*-Gametophyten. Kung Svenska Vet. Akad. Handl. **19**(8): 1–55.

————. 1944. Die Samenbildung und die Zytologie bei agamospermischen und sexuellen Arten von *Elatostema* und einigen nahestehenden Gattungen nebst Beleuchtung einiger damit zusammenhängender Probleme. Kung. Svenska Vet. Akad. Handl. III. **21**(4): 1–130.

FARR, C. H. 1918. Cell division by furrowing in *Magnolia*. Am. Jour. Bot. **5**: 379–395.

FAWORSKY, W. 1906. Nouvelle recherche sur le développement et la cytologie du *Plasmodiophora Brassicae* Woron. Mém. Soc. Nat. Kiew 20. [Russian; French summary].

FEDORTSCHUK, W. 1931. Embryologische Untersuchung von *Cuscuta monogyna* Vahl. und *Cuscuta epithymum* L. Planta **14**: 94–111.

FERGUSON, M. C. 1904. Contributions to the life-history of *Pinus*, with special reference to sporogenesis, the development of the gametophytes, and fertilization. Proc. Wash. Acad. Sci. **6**: 1–202.

FERNALD, M. L. 1918. The diagnostic character of *Vallisneria americana*. Rhodora **20**: 108–110.

FINN, W. W. 1925. Male cells in angiosperms. I. Spermatogenesis and fertilization in *Asclepias cornutii*. Bot. Gaz. **80**: 1–25.

————. 1926. Spermazellen bei *Vincetoxicum*. Ber. Deut. Bot. Ges. **44**: 133–137.

————. 1928*a*. Spermazellen bei *Vinca minor* und *V. herbacea*. Ber. Deut. Bot. Ges. **46**: 235–246.

————. 1928*b*. Über den Pollenschlauch bei *Fagus silvatica*. Nawaschin Fests. 63–66.

————. 1935. Einige Bemerkungen über den männlichen Gametophyten der Angiospermen. Ber. Deut. Bot. Ges. **53**: 679–686.

————. 1936. Zur Entwicklungsgeschichte der Chalazogamen, *Ostrya carpinifolia* Scop. Jour. Inst. Bot. Acad. Sci. RSS. Ukraine **8**: 15–25.

————. 1937*a*. Entwicklungsgeschwindigkeit des männlichen Gametophyten bei den Angiospermen. Trav. Inst. Rech. Sci. Biologie, Univ. Kiew **1937**: 71–86.

————. 1937*b*. Vergleichende Embryologie und Karyologie einiger *Cuscuta*-Arten. Jour. Inst. Bot. Acad. Sci. RSS Ukraine **12**(20) : 83–99.

————. 1939. On the history of the development of the male gametophyte in Labiatae. Jour. Inst. Bot. Acad. Sci. RSS. Ukraine **20**(28) : 77–96.

————. 1940. Spermazellen bei Angiospermen. Jour. Bot. URSS. **25**: 155–175.

————. 1941. Männlichen Gameten bei Angiospermen. Compt. Rend. (Doklady) Acad. Sci. URSS **30**: 451–458.

———— and RUDENKO, T. 1930. Spermatogenesis und Befruchtung bei einigen Orobanchaceae. Bull. Jard. Bot. Kieff **11**: 69–82.

FOSTER, L. T. 1943. Morphological and cytological studies on *Carica papaya*. Bot. Gaz. **105**: 116–126.

FRIEMANN, W. 1910. Über die Entwicklung der generativen Zelle im Pollenkorn der monocotylen Pflanzen. Diss., Bonn.

FRISENDAHL, A. 1912. Cytologische und Entwicklungsgeschichtliche Studien an *Myricaria germanica* Desv. Kung. Svenska Vet. Akad. Handl. **48**(7) : 1–62.

————. 1927. Über die Entwicklung chasmogamer und kleistogamer Blüten bei der Gattung *Elatine*. Acta Hort. Gothoburg **3**: 99–142.

FRYE, T. C. 1902. A morphological study of certain Asclepiadaceae. Bot. Gaz. **34**: 389–413.

————. 1903. The embryo sac of *Casuarina stricta*. Bot. Gaz. **36**: 101–113.

———— and BLODGETT, E. B. 1905. A contribution to the life-history of *Apocynum androsaemifolium*. Bot. Gaz. **40**: 49–53.

FUCHS, A. 1936. Untersuchungen über den männlichen Gametophyten von *Elaeagnus angustifolia*. Öst. Bot. Zeits. **85**: 1–16.

————. 1938. Beiträge zur Embryologie der Thymelaeaceae. Öst. Bot. Zeits. **87**: 1–41.

FULLMER, E. L. 1899. The development of the microsporangia and microspores of *Hemerocallis fulva*. Bot. Gaz. **28**: 80–88.

GAGER, C. S. 1902. The development of the pollinium and sperm cells in *Asclepias cornutii* Decaine. Ann. Bot. **16**: 123–148.

GANGULEE, H. C. 1938. On the development of the embryo-sac and pollen grains in *Canavallia ensiformis* D.C. Jour. Dept. Sci. Calcutta Univ. **1**: 73–94.

GEITLER, L. 1935. Beobachtungen über die erste Teilung im Pollenkorn der Angiospermen. Planta **24**: 361–386.

————. 1937. Zur Morphologie der Pollenkörner von *Clarkia elegans*. Planta **27**: 426–431.

————. 1941. Embryosäcke aus Pollenkörnern bei *Ornithogalum*. Ber. Deut. Bot. Ges. **59**: 419–423.

————. 1942. Über die Struktur des generativen Kerns im zweikernigen Angiospermenpollen. Planta **32**: 187–195.

GELIN, O. E. V. 1934. Embryologische und cytologische Studien in Heliantheae-Coreopsidinae. Acta Horti Bergiani **11**: 99–128.

————. 1936. Zur Embryologie und Zytologie von *Berkheya Bergiana* Söderb. und *B. Adlami* Hook. f. Svensk Bot. Tidskr. **30**: 324–328.

GENTSCHEFF, G. 1937. Zytologische und embryologische Studien über einige *Hieracium*-Arten. Planta **27**: 165–195.

GERASSIMOVA, H. 1933. Fertilization in *Crepis capillaris*. Cellule **42**: 103–148.

GERSHOY, A. 1940. The male gametophytes in some species of violets. Am. Jour. Bot. **27**: 4s.

GOEBEL, K. 1933. Organographie der Pflanzen. III. Samenpflanzen. 3rd ed.

GOLDBERG, B. 1941. Life history of *Peltandra virginica*. Bot. Gaz. **102**: 641–662.

GORE, U. R. 1932. The development of the female gametophyte and embryo in cotton. Am. Jour. Bot. **19**: 795–807.

GRAVES, A. H. 1908. The morphology of *Ruppia maritima*. Trans. Conn. Acad. Sci. **14**: 59–170.

GRIMM, J. 1912. Entwicklungsgeschichtliche Untersuchungen an *Rhus* und *Coriaria*. Flora **104**: 309–334.

GUIGNARD, L. 1889. Études sur les phénomènes morphologiques de la fécondation. Bull. Soc. Bot. France **36**: 100–146.

————. 1891. Nouvelles études sur la fécondation. Ann. Sci. Nat. VII. Bot. **14**: 163–296.

————. 1899. Sur les antherozoides et la double copulation sexuelle chez les végétaux angiospermes. Compt. Rend. Acad. Sci. Paris **128**: 864–871.

————. 1900. L'appareil sexuel et la double fécondation dans les Tulipes. Ann. Sci. Nat. VIII. Bot. **11**: 365–387.

————. 1922. La fécondation et la polyembryonie chez les *Vincetoxicum*. Mém. Acad. Sci. Inst. France. II. **57**: 1–25.

GUILLIERMOND, A. *et al.* 1932. Traité de cytologie végétale.

GURGENOVA, M. 1928. Fertilization in *Phelipaea ramosa*. Nawaschin Fests. 157–168.

GUSTAFSSON, Å. and NYGREN, A. 1946. The temperature effect on pollen formation and meiosis in *Hieracium robustum*. Hereditas **32**: 1–14.

HAGERUP, O. 1938. A peculiar asymmetrical mitosis in the microspore of *Orchis*. Hereditas **24**: 94–96.

————. 1942. The morphology and biology of the *Corylus*-fruit. Kung. Danske Vid. Selskab Biol. Medd. **17**(6): 1–32.

————. 1944. On fertilisation, polyploidy and haploidy in *Orchis maculatus* L. sens. lat. Dansk. Bot. Ark. **11**: 1–26.

————. 1945. Facultative parthenogenesis and haploidy in *Epipactis latifolia*. Kung. Danske Vid. Selskab Biol. Medd. **19**(11): 1–14.

HÅKANSSON, A. 1927. Die Chromosomen einiger Scirpoideen. Hereditas **10**: 277–292.

HALLOCK, F. A. 1930. The relationship of *Garrya*. The development of the flowers and seeds of *Garrya* and its bearing on the phylogenetic position of the genus. Ann. Bot. **44**: 771–812.

HANF, M. 1935. Vergleichende und entwicklungsgeschichtliche Untersuchungen über Morphologie und Anatomie der Griffel und Griffeläste. Beih. Bot. Centralbl. **54A**: 99–141.

HARTIG, TH. 1866. Der Füllkern, der diaphragmatische und der intercellulare Zellkern. Bot. Unters. Physik. Labor. Landw. Lehranst. Berlin, 1: 294.

HEITZ, E. und RÉSENDE, F. 1936. Zur Methodik der Pollenkorn- und Pollenschlauch- Untersuchung. Bol. Soc. Broteriana, Coimbra. II. **11**: 5–15.

HERRIG, F. 1919. Über Spermazellen im Pollenschlauch der Angiospermen. Ber. Deut. Bot. Ges. **37**: 450–453.

————. 1922. Über Fragmentation und Teilung der Pollenschlauchkerne von *Lilium candidum*. Beitr. Allg. Bot. **2**: 403–412.

HEUSSER, K. 1915. Die Entwicklung der generativen Organe von *Himantoglossum hircinum*. Beih. Bot. Centralbl. **32A**: 218–277.

HEWITT, W. C. 1939. Seed development of *Lobelia amoena*. Jour. Elisha Mitchel Sci. Soc. **55**: 63–82.

HOARE, G. 1934. Gametogenesis and fertilization in *Scilla nonscripta*. Cellule **42**: 269–292.

HOFMEISTER, W. 1847. Untersuchungen des Vorganges bei der Befruchtung der Oenotheren. Bot. Zeit. **5**: 785–792.

————. 1849. Die Entstehung des Embryo der Phanerogamen.

————. 1852. Zur Entwicklungsgeschichte der *Zostera*. Bot. Zeit. **10**: 121–131; 137–149.

————. 1858. Neuere Beobachtungen über Embryobildung der Phanerogamen. Jahrb. Wiss. Bot. **1**: 82-190.

————. 1861. Neue Beiträge zur Kenntnis der Embryobildung der Phanerogamen. II. Monokotyledonen. Abh. Math.-Physik. Kön. Sächs. Ges. Wiss. **7**: 629–760.

ISHIKAWA, M. 1918. Studies on the embryo sac and fertilization in *Oenothera*. Ann. Bot. **32**: 279–317.

IYENGAR, N. K. 1938. Pollen-tube studies in *Gossypium*. Jour. Genet. **37**: 69–106.

JOHANSEN, D. A. 1936. Morphology and embryology of *Fouquieria*. Am. Jour. Bot. **23**: 95–99.

JOHNSTON, G. W. 1941. Cytological studies of male gamete formation in certain angiosperms. Am. Jour. Bot. **28**: 306–319.

JOHRI, B. M. 1934. The development of the male and female gametophytes in *Cuscuta reflexa* Roxb. Proc. Indian Acad. Sci. B. **1**: 283–289.

————. 1936*a*. Studies in the family Alismaceae. IV. *Alisma plantago* L.; *A. plantago-aquatica* L.; and *Sagittaria graminea* Mich. Proc. Indian Acad. Sci. B. **4**: 128–138.

————. 1936*b*. The life history of *Butomopsis lanceolata* Kunth. Proc. Indian Acad. Sci. B. **4**: 139–162.

JONES, H. A. 1927. Pollination and life history studies of Lettuce (*Lactuca sativa*). Hilgardia **2**: 425–442.

JOSHI, A. C. 1946. A note on the development of pollen of *Myristica fragrans* Van Houtten and the affinities of the family Myristicaceae. Jour. Indian Bot. Soc. **25**: 140–143.

———— and KAJALE, L. B. 1937. Fertilisation and seed development in Amarantaceae. Proc. Indian Acad. Sci. B. **5**: 91–100.

JOSHI, P. C. 1936. Contribution to the life history of *Stellaria media* L. Proc. Indian Acad. Sci. B. **3**: 8–22.

JOST, L. 1905. Zur Physiologie des Pollens. Ber. Deut. Bot. Ges. **23**: 504–505.

————. 1907. Über die Selbsterilität einiger Blüten. Bot. Zeit. **65**: 77–117.

JUEL, H. O. 1900. Beiträge zur Kenntnis der Tetradenteilung. Jahrb. Wiss. Bot. **35**: 626–659.

————. 1902–1903. Zur Entwicklungsgeschichte des Samens von *Cynomorium*. Beih. Bot. Centralbl. **13**: 194–202.

———. 1907. Studien über die Entwicklungsgeschichte von *Saxifraga granulata*. Nova Acta Reg. Soc. Sci. Upsalensis, IV. 1(9): 1–41.

Jüssen, F. J. 1928. Die Haploidgeneration der Araceen und ihre Verwertung für das System. Bot. Jahrb. **62**: 193–283.

Juliano, J. B. 1935. Anatomy and morphology of the Bunga, *Aeginetia indica* L. Philippine Jour. Sci. **56**: 405–451.

——— and Alcala, P. E. 1933. Floral morphology of *Musa errans* (Blanco) Teodoro var. *Botoan* Teodoro. Philippine Agr. **22**: 91–126.

Junell, S. 1931. Die Entwicklungsgeschichte von *Circaeaster agrestis*. Svensk Bot. Tidskr. **25**: 238–270.

Kadry, A. E. R. 1946. Embryology of *Cardiospermum Halicacabum* L. Svensk Bot. Tidskr. **40**: 111–126.

Karsten, G. 1891. Über die Mangrove-Vegetation im malayischen Archipel. Bibl. Bot. **22**.

———. 1902. Über die Entwicklung der weiblichen Blüten bei einiger Juglandaceen. Flora **90**: 316–333.

Katz, E. 1926. Über die Funktion der Narbe bei der Keimung des Pollens. Flora **120**: 243–273.

Katz, E. J. 1943. Pollen tube growth in *Taraxacum officinale*. Bot. Gaz. **104**: 650.

———. 1938. Pollen development and seed formation in *Utricularia coerulea* L. Beih. Bot. Centralbl. **58A**: 365–378.

Kausik, S. B. and Rao, P. V. K. 1942. The male gametophyte of *Halophila ovata* Gaudich. Half-yearly Jour. Mysore Univ. B. **3**: 43–49.

Kearney, T. H. 1923. Self-fertilization and cross-fertilization in Pima cotton. U. S. Dept. Agr., Bull. 1134.

Khan, R. 1942. A contribution to the embryology of *Jussieua repens* Linn. Jour. Indian Bot. Soc. **21**: 267–282.

Kirkwood, J. C. 1906. The pollen tube in some of the Cucurbitaceae. Bull. Torrey Bot. Club **33**: 327–342.

Körnicke, M. 1906. Zentrosomen bei Angiospermen? Zugleich ein Beitrag zur Kenntnis der generativen Elemente im Pollenschlauch. Flora **96**: 501–522.

Kostriukova, K. 1939a. Spermatogenesis in *Crinum Hildebrandtii* Vatke. Observations *in vivo*. Jour. Inst. Bot. Acad. Sci. RSS. Ukraine **21/22**: 157–164.

———. 1939*b*. Observations in vivo on the formation of the male sex cells in *Lilium Martagon* L. Compt. Rend. (Doklady) Acad. Sci. URSS **22**: 444–447.

———. 1941. On the pellicular layer of the cytoplasm of the generative cell of *Convallaria majalis* L. Compt. Rend. (Doklady) Acad. Sci. URSS **30**: 463–465.

———. 1945. Le gametophyte mâle des amaryllidees. Soviet Bot. **13**: 32–48.

——— and Benetskaia, G. 1939. Spermatogenesis in *Narcissus poeticus* L. Observations *in vivo*. Jour. Bot. URSS **24**: 209–220.

Krishna Iyengar, C. V. 1940. Development of the embryo sac and endosperm haustoria in some members of the Scrophularineae. IV. *Vandellia hirsuta* Ham. and *V. scabra* Benth. Jour. Indian Bot. Soc. **18**: 179–189.

———. 1941. Development of the embryo sac and endosperm haustoria in *Torenia cordifolia* Roxb. and *T. hirsuta* Benth. Proc. Nat. Inst. Sci. India **7**: 61–71.

Krug, C. A. and Mendes, A. J. T. 1940. Cytological observations in *Coffea*. IV. Jour. Genet. **39**: 189–203.

Krupko, S. 1926. Les plastides et le chondriome pendant la gonogenèse dans le *Gagea lutea* L. Acta Soc. Bot. Poloniae **4**: 77–86.

———. 1944. On the sterility of *Oxalis cernua* in the Mediterranean. Przyroda **1944**: 1–32.

LAGERBERG, T. 1909. Studien über die Entwicklungsgeschichte und systematische Stellung von *Adoxa moschatellina* L. Kung. Svenska Vet. Akad. Handl. **44**(4): 1–86.

LAKSHMINARASIMHA MURTY, K. 1938. Gametogenesis and embryogeny in some Commelinaceae. Jour. Indian Bot. Soc. **17**: 101–116.

LAND, W. J. G. 1900. Double fertilization in Compositae. Bot. Gaz. **30**: 252–260.

LANGDON, LA DEMA M. 1934. Embryogeny of *Carya* and *Juglans*, a comparative study. Bot. Gaz. **96**: 93–117.

LEBON, E. 1929. Sur la formation de l'albumen chez *Impatiens Sultani*. Compt. Rend. Soc. Biol. (Paris) **101**: 1168–1170.

LEITNER, J. 1942. Ein Beitrag zur Kenntnis der Pollenkörner der Labiatae. Öst. Bot. Zeits. **91**: 29–40.

LEVAN, A. 1939. Abnorm utformning av pollen inon ett pollenfack av. *Beta*. Bot. Not. **1939**: 363–365.

————. 1941. Syncyte formation in the pollen mother-cells of haploid *Phleum pratense*. Hereditas **27**: 243–252.

LIASCHCHENKO, L. T. 1940. Cytoplasmic heredity in sunflower. Compt. Rend. (Doklady) Acad. Sci. URSS **27**: 823–824.

LOCKE, J. F. 1936. Microsporogenesis and cytokinesis in *Asimina triloba*. Bot. Gaz. **98**: 159–168.

LONGO, B. 1901. La mesogamia nella commune Zucca (*Cucurbita Pepo* L.). Rend. R. Accad. Lincei Roma. V. **10**: 168–172.

————. 1903. La nutrizione dell'embrione delle *Cucurbita* operate per mezzo del tubetto pollinico. Ann. Bot. (Roma) **1**: 71–74.

MADGE, M. 1929. Spermatogenesis and fertilisation in the cleistogamous flowers of *Viola odorata* var. *praecox*. Ann. Bot. **43**: 545–577.

————. 1936. Division of the generative cell in *Hedychium gardnerianum*. Cellule **45**: 171–176.

MAHESHWARI, P. 1929. Contribution to the morphology of *Boerhaavia diffusa*. I. Jour. Indian Bot. Soc. **8**: 219–234.

————. 1931. Contribution to the morphology of *Albizzia lebbek*. Jour. Indian Bot. Soc. **10**: 241–264.

————. 1945. The place of angiosperm embryology in research and teaching. Jour. Indian Bot. Soc. **24**: 25–41.

————. 1946. The Adoxa type of embryo sac: a critical review. Lloydia **9**: 73–113.

————. 1947. Tetranucleate embryo sacs in angiosperms. Lloydia **10**: 1-18. 1947.

————. 1948. The angiosperm embryo sac. Bot. Rev. **14**: 1–56. 1948.

———— and JOHRI, B. M. 1941. The embryo sac of *Acalypha indica* L. Beih. Bot. Centralbl. **61A**: 125–136.

———— and SINGH, BAHADUR. 1934. A preliminary note on the morphology of the aerial and underground flowers of *Commelina benghalensis* Linn. Curr. Sci. **3**: 158–160.

———— and SINGH, BALWANT. 1943. Studies in the family Alismaceae. V. The embryology of *Machaerocarpus californicus* (Torr.) Small. Proc. Nat. Inst. Sci. India **9**: 311–322.

———— and WULFF, H. D. 1937. Recent advances in microtechnic. I. Methods of studying the male gametophyte of angiosperms. Stain Tech. **12**: 61–70.

MAHONY, K. L. 1935. Morphological and cytological studies on *Fagopyrum esculentum*. Am. Jour. Bot. **22**: 460–475.

MANSHARD, E. 1936. Embryologische Untersuchungen an *Styrax obassia* Sieb. et Zucc. Planta **25**: 364–383.

MARQUARDT, H. and ERNST, H. 1939/40. Zytogenetische Röntgenversuche am reifenden Pollen von *Antirrhinum majus*. Zeits. Bot. **35**.

MARTIN, J. N. 1913. The physiology of pollen of *Trifolium pratense*. Bot. Gaz. **56**: 112–126.

MATSUDA, H. 1930. Further studies on the origin of giant pollen grains in *Petunia*. Proc. Corp. Sci. Soc. Japan **2**: 2.

MAURITZON, J. 1933. Studien über die Embryologie der Familien Crassulaceae und Saxifragaceae. Akad. Abh. Lund; pp. 152.

———. 1939. Contributions to the embryology of the orders Rosales and Myrtales. Lunds Univ. Arsskrift N. F. Avd. **2**, 35: 1–120.

MILLER, E. C. 1919. Development of the pistillate spikelet and fertilization in *Zea mays* L. Jour. Agr. Res. **18**: 255–266.

MOFFETT, A. A. 1932. Studies on the formation of multinuclear giant pollen grains in *Kniphofia*. Jour. Genet. **25**: 315–337.

MOHRBUTTER, C. 1936. Embryologische Studien an Loganiaceen. Planta **26**: 64–80.

MÜLLER, H. 1936. Zytologische Untersuchungen über die Haploidgeneration der Apocynaceen.

MURBECK, S. 1901. Über das Verhalten des Pollenschlauches bei *Alchemilla arvensis* und das Wesen der Chalazogamie. Lunds Univ. Arsskr. N. F. Avd. **2**, 9: 1–46.

———. 1902. Über die Embryologie von *Ruppia rostellata* Koch. Kungl. Svenska Vetensk. Akad. Handl. **36**: 1–21.

NAGOUCHI, Y. 1929. Zur Kenntnis der Befruchtung und Kornbildung bei den Reispflanzen. Jap. Jour. Bot. **4**: 385–403.

———. 1931. Studien über Reispflanzen. II. Pollenkeimung und Pollenschlauchwachstum. Jap. Jour. Bot. **5**: 351–368.

NAITHANI, S. P. 1937. Chromosome studies in *Hyacinthus orientalis* L. III. Reversal of sexual state in the anthers of *H. orientalis* L. var. Yellow Hammer. Ann. Bot. **1**: 369–377.

NARASIMHA MURTHY, S. K. 1935. The life history of *Ottelia alismoides* Pers. Proc. Indian Acad. Sci. B. **2**: 59–66.

NAST, C. G. 1941. The embryogeny and seedling morphology of *Juglans regia* L. Lilloa **6**: 163–205.

NAWASCHIN, S. G. 1897. Über die Befruchtung bei *Juglans regia* und *J. nigra*. Trav. Soc. Imp. Nat. St. Pétersbourg **28**.

———. 1898. Resultate einer Revision der Befruchtungsvorgänge bei *Lilium Martagon* und *Fritillaria tenella*. Bull. Acad. Imp. Sci. St. Pétersbourg **9**: 377–382.

———. 1900. Über die Befruchtungsvorgänge bei einigen Dicotyledonen. Ber. Deut. Bot. Ges. **18**: 224–230.

———. 1909. Über dasselbständige Bewegungsvermögen des Spermakerns bei einigen Angiospermen. Öst. Bot. Zeits. **59**: 457–467.

———. 1910. Näheres über die Bildung der Spermakerne bei *Lilium Martagon*. Ann. Jard. Bot. Buitenzorg, 3rd Suppl. **2**: 871–904.

——— and FINN, W. W. 1912. Zur Entwicklungsgeschichte der Chalazogamen, *Juglans regia* und *Juglans nigra*. Mem. Acad. Sci. St. Pétersbourg VIII, **31**: 1–59.

NEGODI, G. 1936. Cariologia delle specie italiane del genre *Bellis* e contributo all'apprezzamento dello stato energetico dei genomi di specie omoploidi ed eteroplodi. Biol. Gen. (Wien) **12**: 546–558.

NĚMEC, B. 1898. Über den Pollen der petaloiden Antheren von *Hyacinthus orientalis* L. Rozpravy Česke Akad. Prag II, **7**(17).

———. 1931. Fecundation in *Gagea lutea*. Preslia **10**: 104–110.

NEWCOMBE, F. C. 1924. Significance of the behavior of sensitive stigmas. II. Am. Jour. Bot. **11**: 85–93.

NEWMAN, I. V. 1934. Studies in the Australian acacias. IV. The life history of *Acacia Baileyana* F. V. M. Part 2. Gametophytes, fertilization, seed production and germination, and general conclusion. Proc. Linn. Soc. New South Wales **59**: 277–313.

Nohara, S. 1934. Gametogenesis and embryogeny of *Sesamum indicum* L. Jour. Coll. Agr. Tokyo Imp. Univ. 13: 19–25.

Nothnagel, M. 1918. Fecundation and formation of the primary endosperm nucleus in certain Liliaceae. Bot. Gaz. 66: 141–161.

Oehler, E. 1927. Entwicklungsgeschichtlich-cytologische Untersuchungen an einigen saprophytischen Gentianaceen. Planta 3: 641–733.

Ohga, I. 1937. On the fertilization of *Nelumbo nucifera*. Cytologia, Fujii Jubl. Vol.: 1033–1035.

Oksijuk, P. 1927. Entwicklungsgeschichte der Zuckerrübe (*Beta vulgaris*). Bull. Jard. Bot. Kieff 5/6: 145–164.

———. 1934. On the biology of blossoming in the sugar beet. Jour. Inst. Bot. Acad. Sci. RSS. Ukraine 9: 75–84.

O'Mara, J. 1933. Division of the generative nucleus in the pollen tube of *Lilium*. Bot. Gaz. 94: 567–578.

Osawa, J. 1912. Cytological and experimental studies on *Citrus*. Jour. Coll. Agr. Tokyo Imp. Univ. 4: 83–116.

———. 1913. On the development of the pollen-grain and embryo-sac of *Daphne*, with special reference to the sterility of *Daphne odora*. Jour. Coll. Agr. Imp. Univ. Tokyo 4: 237–264.

Pace, L. 1907. Fertilization in *Cypripedium*. Bot. Gaz. 44: 353–374.

Paetow, W. 1931. Embryologische Untersuchungen an Taccaceen, Meliaceen und Dilleniaceen. Planta 14: 441–470.

Pastrana, M. D. and Santos, J. K. 1931. A contribution on the life history of *Dendrobium anosmum* Lindley. Nat. & Appl. Sci. Bull., Univ. Philippines 1: 133–144.

Paton, J. B. 1921. Pollen and pollen enzymes. Am. Jour. Bot. 8: 471–501.

Pearson, O. H. 1933. Study of the life-history of *Brassica oleracea*. Bot. Gaz. 94: 534–550.

Penland, C. W. T. 1923. Cytological behavior in *Rosa*. Bot. Gaz. 76: 403–410.

Persidsky, D. 1926. Zur Embryologie der *Orobanche cumana* Wallr. und der *O. ramosa* L. Bull. Jard. Bot. Kieff 4: 6–10.

———. 1940. Embryological and cytological investigation of barley (*Hordeum distichum* L.). Jour. Inst. Bot. Acad. Sci. RSS. Ukraine 1: 145–153.

Peter, K. 1930. Die Beziehungen zwischen Zellteilung und Zelltätigkeit, Darstellung und Versuch einer kausalen Betrachtung. Sammelreferat. Protoplasma 10: 613–625.

Piech, K. 1924*a*. Zur Entwicklung der Pollenkörner bei *Scirpus lacustris*. Bull. Acad. Polon. Math.-Nat. Cracovie, B: 113–123.

———. 1924*b*. Über die Teilung des primären Pollenkerns und die Entstehung der Spermazellen bei *Scirpus paluster*. Bull. Acad. Polon. Math.-Nat. Cracovie, B: 605–621.

———. 1928. Über die Entstehung der generativen Zelle bei *Scirpus uniglumis* Link. durch "freie Zellbildung". Planta 6: 96–117.

Poddubnaja-Arnoldi, V. A. 1927. Spermatogenesis bei einigen Compositen. Planta 4: 284–298.

———. 1933. Künstliche Kultur und zytologische Untersuchung des Pollenschlauches vom *Senecio platanifolius* Benth. Planta 19: 299–304.

———. 1934. Spermazellen in der Familie der Dipsacaceae. Planta 21: 381–386.

———. 1936. Beobachtungen über die Keimung des Pollens einiger Pflanzen auf künstlichen Nährboden. Planta 25: 502–529.

——— and Dianowa, V. 1934. Eine zytoembryologische Untersuchung einiger Arten der Gattung *Taraxacum*. Planta 23: 19–46.

Pope, M. N. 1937. The time factor in pollen tube growth and fertilization in barley. Jour. Agr. Res. 54: 525–529.

———. 1943. The temperature factor in fertilization and growth of barley. Jour. Agr. Res. **66**: 389–402.

———. 1946. The course of the pollen tube in cultivated barley. Jour. Am. Soc. Agron. **38**: 432–440.

Popham, R. A. 1938. A contribution to the life history of *Galinsoga ciliata*. Bot. Gaz. **99**: 543–555.

Raghavan, T. S. 1937. Studies in the Capparidaceae. I. The life history of *Cleome Chelidonii* Linn. Jour. Linn. Soc. London, Bot. **51**: 43–72.

——— *et al.* 1939. Division of the generative cell in *Impatiens Balsamina* L. Cytologia **9**: 389–392.

Rangasami, K. 1935. On the cytology of *Pennisetum typhoideum* Rich. Jour. Indian Bot. Soc. **14**: 125–131.

Rao, L. N. 1942. Studies in the Santalaceae. Ann. Bot. **6**: 151–175.

Rauch, K. V. 1936. Cytologisch-embryologische Untersuchungen an *Scurrula atropurpurea* Dans. und *Dendrophthoe pentandra* Miq. Ber. Schweiz. Bot. Ges. **45**: 5–61.

Reeves, R. G. 1930. Development of the ovule and embryo sac of alfalfa (*Medicago sativa* L.). Am. Jour. Bot. **17**: 239–246.

Reichenbach, H. G. 1852. De pollinis Orchidearum genesi ac structura et de Orchideis in artem ac systema regigendis.

Résende, F. 1941. Vielkernige Mikrosporem bei einer Rasse von *Antirrhinum majus* L. Boletim Soc. Broteriana, Coimbra II, **15**: 5–7.

Rocén, Th. 1927. Zur Embryologie der Centrospermen. Diss. Uppsala.

Rosenberg, O. 1901. Über die Pollenbildung von *Zostera*. Meddel. Stockholma Högskolas Bot. Inst.: 1–21.

Rudenko, T. 1930. Male cells of Scrophulariaceae. Spermatogenesis and fertilization by *Lathraea squamaria*. Bull. Jard. Bot. Kieff **11**: 41–45.

Ruhland, W. and Wetzel, K. 1924. Der Nachweis von Chloroplasten in den generativen Zellen von Pollenschläuchen. Ber. Deut. Bot. Ges. **42**: 3–14.

Safijovska, L. D. 1935. Zur Embryologie der *Adenophora lillifolia* Led. Jour. Inst. Bot. Acad. Sci. RSS. Ukraine **3**(11): 85–98.

Sakai, K. 1939. A cytological examination of the decreasing fertility of cultivated rice in Northern Japan. Proc. Crop Sci. Soc. Japan **11**: 40–49. [Japanese, English sum.]

Samuels, J. A. 1912. Études sur le développement du sac embryonnaire et sur la fécondation du *Gunnera macrophylla* Bl. Arch. Zellf. **8**: 52–120.

Samuelsson, G. 1913. Studien über die Entwicklungsgeschichte einiger Bicornes-Typen. Svensk Bot. Tidskr. **7**: 97–188.

———. 1914. Über die Pollenentwicklung von *Anona* und *Aristolochia* und ihre systematische Bedeutung. Svensk Bot. Tidskr. **8**: 181–189.

Santos, J. K. 1940. The origin and development of the female gametophyte of *Hedychium coronarium* Koenig. Nat. & Appl. Sci. Bull. Univ. Philippines **7**: 245–268.

Sanz, C. 1945. Pollen-tube growth in intergeneric pollinations on *Datura stramonium*. Proc. Nat. Acad. Sci. **31**: 361–367.

Sawyer, M. L. 1917. Pollen tubes and spermatogenesis in *Iris*. Bot. Gaz. **64**: 159–164.

———. 1925. Crossing *Iris pseudacorus* and *I. versicolor*. Bot. Gaz. **79**: 60–72.

Sax, K. 1916. Fertilization in *Fritillaria pudica*. Bull. Torrey Bot. Club **43**: 505–522.

———. 1918. The behavior of the chromosomes in fertilization. Genetics **3**: 309–327.

———. 1935. The effect of temperature on nuclear differentiation in microspore development. Jour. Arn. Arb. **16**: 301–310.

——— and Edmonds, H. W. 1933. Development of the male gametophyte in *Tradescantia*. Bot. Gaz. **95**: 156–163.

——— and Husted, L. 1936. Polarity and differentiation in microspore development. Am. Jour. Bot. **23**: 606–609.

——— and O'Mara, J. 1941. Mechanism of mitosis in pollen tubes. Bot. Gaz. **102**: 629–636.

Schaeppi, H. and Steindl, F. 1942. Blütenmorphologische und embryologische Untersuchungen an Loranthoideen. Vierteljahrschr. Naturf. Ges. Zürich **87**: 301–372.

Schaffner, J. H. 1897. The development of the stamens and carpels of *Typha latifolia*. Bot. Gaz. **24**: 93–102.

———. 1901. A contribution to the life history and cytology of *Erythronium*. Bot. Gaz. **31**: 369–387.

Schnarf, K. 1929. Embryologie der Angiospermen.

———. 1931. Vergleichende Embryologie der Angiospermen.

———. 1933. Embryologie der Gymnospermen.

———. 1937. Studien über den Bau der Pollenkörner der Angiospermen. Planta **27**: 450–465.

———. 1939. Variation im Bau des Pollenkörnes der Angiospermen. Tab. Biol. **17**: 72–89.

———. 1941. Vergleichende Zytologie des Geschlechtsapparates des Kormophyten. Monographien zur vergleichenden Zytologie.

——— and Wunderlich, R. 1939. Zur vergleichende Embryologie der Liliaceae-Asphodeloideae. Flora **33**: 297–327.

Schürhoff, P. N. 1921. Über die Teilung des generativen Kernes vor der Keimung des Pollenkorns. Archiv Zellf. **15**: 145–159.

———. 1926. Die Zytologie der Blütenpflanzen.

Sears, E. R. 1937. Cytological phenomena connected with self-sterility in flowering plants. Genetics **22**: 130–181.

Sexsmith, J. J. and Fryer, J. R. 1943. Studies relating to fertility in alfalfa (*Medicago sativa* L.). I and II. Sci. Agr. **24**: 95–100; 145–151.

Shattuck, C. H. 1905. A morphological study of *Ulmus americana*. Bot. Gaz. **40**: 209–223.

Shibata, K. 1902. Die Doppelbefruchtung bei *Monotropa uniflora*. Flora **90**: 61–66.

Shoemaker, D. N. 1905. On the development of *Hamamelis virginiana*. Bot. Gaz. **39**: 245–266.

Shoemaker, J. S. 1926. Pollen development in the apple, with special reference to chromosome behavior. Bot. Gaz. **81**: 148–172.

Smith, F. H. 1942. Development of the gametophytes and fertilization in *Camassia*. Am. Jour. Bot. **29**: 657–663.

Smith, Ora. 1935. Pollination and life history studies of the tomato (*Lycopersicon esculentum* Mill.). Cornell Univ. Agr. Exp. Sta., Mem. **184**: 1–16.

——— and Cochran, H. L. 1935. Effect of temperature on pollen germination and tube growth in the tomato. Cornell Univ. Agr., Exp. Sta. Mem. 175: 1–11.

Smith, R. W. 1898. A contribution to the life history of the Pontederiaceae. Bot. Gaz. **40**: 324–338.

Souèges, E. C. R. 1919. Recherches sur l'embryogénie des Polygnoacées. Bull. Soc. Bot. France **66**: 168–199.

Sprague, G. F. 1932. The nature and extent of hetero-fertilization in maize. Genetics **17**: 358–368.

Srinivasa Iyengar, G. 1937. Life history of *Santalum album* Linn. Jour. Indian Bot. Soc. **16**: 175–195.

Stejskal-Streit, Vilma. 1940. Vergleichende Untersuchungen gehemter Staubblätter. Öst. Bot. Zeits. **89**: 1–56.

Stenar, H. 1925. Embryologische Studien. I. Zur Embryologie der Columniferen. II. Die Embryologie der Amaryllideen. Akad. Abh. Uppsala.

————. 1937. Zur Embryosackentwicklung einiger Malpighiazeen. Bot. Not. 1937: 110–118.

Stephens, J. C. and Quinby, J. C. 1934. Anthesis, pollination and fertilization in sorghum. Jour. Agr. Res. 49: 123–136.

Stern, H. 1946. The formation of polynucleated pollen mother cells. Jour. Hered. 37: 47–50.

Stevens, N. 1912. Observations on heterostylous plants. Bot. Gaz. 53: 277–308.

Stout, A. B. 1912. The individuality of the chromosomes and their serial arrangements in *Carex aquatilis*. Archiv. Zellf. 9: 114–140.

————. 1916. Self- and cross-pollinations in *Cichorium Intybus* with reference to sterility. Mem. N. Y. Bot. Gard. 6: 333–454.

————. 1931. Pollen-tube behavior in *Brassica pekinensis* with reference to self-incompatibility in fertilization. Am. Jour. Bot. 18: 686–695.

————. 1938. The genetics of incompatibilities in homomorphic flowering plants. Bot. Rev. 4: 275–369.

Stow, I. 1930. Experimental studies on the formation of the embryo sac-like giant pollen grains in the anther of *Hyacinthus orientalis*. Cytologia 1: 417–439.

————. 1934. On the female tendencies of the embryo sac-like giant pollen grain of *Hyacinthus orientalis*. Cytologia 5: 88–108.

Strasburger, E. 1877. Über Befruchtung und Zellteilung.

————. 1884. Neuere Untersuchungen über den Befruchtungsvorgang bei den Phanerogamen als Grundlage für eine Theorie der Zeugung.

————. 1886. Über fremdartige Bestäubung. Jahrb. Wiss. Bot. 17: 50–98.

————. 1908. Chromosomenzahlen, Plasmastrukturen, Verebungsträger und Reduktionsteilung. Jahrb. Wiss. Bot. 45: 477–570.

Subba Rao, A. M. 1940. Studies in the Malpighiaceae. I. Embryo sac development and embryogeny in the genera *Hiptage, Banisteria* and *Stigmatophyllum*. Jour. Indian Bot. Soc. 18: 145–156.

————. 1941. Studies in the Malpighiaceae. II. Structure and development of the ovules and embryo sacs of *Malpighia coccifera* Linn. and *Tristellateia australis*. Proc. Nat. Inst. Sci. India 7: 393–404.

Subramanyam, K. 1942. Gametogenesis and embryogeny in a few members of the Melastomaceae. Jour. Indian Bot. Soc. 21: 69–85.

Suessenguth, K. 1923. Über die Pseudogamie bei *Zygopetalum Mackayi* Hook. Ber. Deut. Bot. Ges. 41: 16–23.

Suita, N. 1936. Studies on the male gametophyte in Angiosperms. I. Studies in the degenerating process of pollen tube nucleus, with special reference to Feulgen's reaction. Bot. & Zool. (Tokyo) 4: 2033–2044. [Japanese, Eng. summ.]

————. 1937*a*. On the mature pollen grains in angiosperms. Bot. Mag. Tokyo 51: 524–530.

————. 1937*b*. Studies on the male gametophyte in angiosperms. II. Differentiation and behavior of the vegetative and generative elements in the pollen grains of *Crinum*. Cytologia, Fujii Jubl. Vol.: 920–933.

————. 1938. Studies on the male gametophyte in angiosperms. IV. Behavior of the "droplets-sheath" in the pollen tube. Cytologia 8: 532–541.

————. 1939*a*. Studies on the male gametophyte in angiosperms. V. Colchicine treatment as a proof of the essential function of the spindle mechanism in karyokinesis in the pollen tube. Jap. Jour. Genet. 15: 91–95.

————. 1939*b*. Studies on the male gametophyte in angiosperms. VI. Abnormalities in the pollen development of some *Crinum*-species. Jap. Jour. Genet. 15: 209–224. [Japanese, Eng. summ.]

————. 1939*c*. Karyokinesis and cytokinesis in the pollen tube. Jap. Jour. Genet. 15: [Japanese, Eng. summ.]

Swamy, B. G. L. 1944. A preliminary note on the embryology of *Casuarina equisetifolia* Forst. Proc. Indian Acad. Sci. B. 20: 187–191.

————. 1946*a*. The embryology of *Zeuxine sulcata* Lindl. New Phytol. 45: 132–136.

————. 1946*b*. Embryology of *Habenaria*. Proc. Nat. Inst. Sci. India 12: 413–426.

Täckholm, G. 1915. Beobachtungen über die Samenentwicklung einiger Onagraceen. Svensk Bot. Tidskr. 9: 294–361.

Tanaka, N. 1939*a*. Chromosome studies in Cyperaceae. IV. Pollen development of *Carex grallatoria* Maxim. var. *heteroclita* Kukenth. ex Matsum. Jap. Jour. Genet. 15: 153–157.

————. 1939*b*. Chromosome number and pollen development of *Fimbristylis*. Bot. Mag. Tokyo 53: 480–488.

————. 1940. Chromosome studies in Cyperaceae. VI. Pollen development and additional evidence for the compound chromosome in *Scirpus lacustris* L. Cytologia 10: 348–362.

————. 1941. Chromosome studies in Cyperaceae. XII. Pollen development in five genera, with special reference to *Rhyncospora*. Bot. Mag. Tokyo 55: 55–65.

Thomas, P. T. 1942. A useful abnormality of the pollen in a pear. Nature 149: 168–169.

Thompson, R. C. 1933. A morphological study of flower and seed development in cabbage. Jour. Agr. Res. 47: 215–232.

Tischler, G. 1913. Über die Entwicklung der Samenanlagen in parthenocarpen Angiospermenfrüchten. Jahrb. Wiss. Bot. 52: 1–84.

————. 1942/43. Allgemeine Pflanzenkaryologie. Vol. II.

Tokugawa, Y. 1914. Zur Physiologie des Pollens. Jour. Coll. Sci. Tokyo Imp. Univ. 35: 1–35.

Trankowsky, D. A. 1931. Zytologische Beobachtungen über die Entwicklung der Pollenschläuche einiger Angiospermen. Planta 12: 1–18.

————. 1938. La spermogenèse et la fécondation chez la Drosère (*Drosera*). Bull. Soc. Nat. Moscou, Sect. Biol. 47: 104–112.

————. 1939. Sur la division du noyau générateur dans le tube pollinique des angiospermes. Bull. Soc. Nat. Moscou, Sect. Biol. 48: 125–129.

Treub, M. 1891. Sur les Casuarinées et leur place dans le système naturel. Ann. Jard. Bot. Buitenzorg 10: 145–231.

Tschernojarow, M. W. 1926. Befruchtungserscheinungen bei *Myosurus minimus*. Öst. Bot. Zeits. 75: 197–206.

Umiker, O. 1920. Entwicklungsgeschichtlich-zytologische Untersuchungen an *Helosis guayensis* Rich. Diss., Zurich.

Upcott, M. 1936. The mechanism of mitosis in the pollen-tube of *Tulipa*. Proc. Royal Soc. London B. 121: 207–220.

————. 1939. The external mechanics of the chromosomes. VII. Abnormal mitosis in the pollen-grains. Chromosoma 1: 178–190.

Van Tieghem, Ph. 1869. Recherches physiologiques sur la vegetation libre du pollen. Ann. Sci. Nat. V. Bot. 12: 312–328.

Venkateswarlu, J. 1937. A contribution to the embryology of Sonneratiaceae. Proc. Indian Acad. Sci. B. 5: 206–223.

————. 1945. Embryological studies in the Thymelaeaceae. Jour. Indian Bot. Soc. 24: 45–66.

Vignoli, L. 1936. Cariologia del genre *Agave*. Lavori Inst. Bot. Palermo 7: 176.

————. 1937. Cariologia del genre *Agave*. Lavori Inst. Bot. Palermo 8: 1.

Wanscher, J. H. 1939. Contribution to the cytology and life history of apple and pear. Royal Vet. & Agr. Coll., Copenhagen, Yearbook 1939: 21–70.

WARMKE, H. E. 1943. Macrosporogenesis, fertilization, and early embryology of *Taraxacum kok-saghys*. Bull. Torrey Bot. Club **70**: 164–173.

WEBER, E. 1929. Entwicklungsgeschichtliche Untersuchungen über die Gattung *Allium*. Bot. Archiv **25**: 1–44.

WEFELSCHEID, G. 1911. Über die Entwicklung der generativen Zelle im Pollenkorn der dikotylen Angiospermen. Diss., Bonn.

WEINSTEIN, A. J. 1926. Cytological studies on *Phaseolus vulgaris*. Am. Jour. Bot. **13**: 248–263.

WEINZIEHER, S. 1914. Beiträge zur Entwicklungsgeschichte von *Xyris indica* L. Flora **106**: 393–432.

WELSFORD, E. J. 1914. The genesis of the male nuclei in *Lilium*. Ann. Bot. **28**: 265–270.

WENIGER, W. 1918. Fertilization in *Lilium*. Bot. Gaz. **66**: 259–268.

WERNER, E. 1914. Zur Ökologie atypischer Samenanlagen. Beih. Bot. Centralbl. **32**: 1–14.

WEST, G. 1930. Cleistogamy in *Viola Riviniana*, with especial reference to the cytological aspects. Ann. Bot. **44**: 87–109.

WILLE, N. 1886. Über die Entwicklungsgeschichte der Pollenkörner der Angiospermen und das Wachstum der Membranen durch Intussusception. Christiania Videnskabsselskabs Forhandl. Nr. 5.

WILSON, G. B. 1946. Cytological studies in the Musae. I. Meiosis in some triploid clones. Genetics **31**: 241–258.

WITMER, S. W. 1937. Morphology and cytology of *Vallisneria spiralis* L. Am. Mid. Nat. **18**: 309–327.

WOLPERT, J. 1910. Vergleichende Anatomie und Entwicklungsgeschichte von *Alnus alnobetula* und *Betula*. Flora **100**: 37–67.

WOODROOF, J. G. and WOODROOF, N. C. 1927. The development of the pecan-nut (*Hicoria pecan*) from the flower to the maturity. Jour. Agr. Res. **34**: 1049–1063.

WOODROOF, N. C. 1928. Development of the embryo sac and young embryo in *Hicoria pecan*. Am. Jour. Bot. **15**: 416–421.

WULFF, H. D. 1933. Beiträge zur Kenntnis des männlichen Gametophyten der Angiospermen. Planta **21**: 12–50.

———. 1934. Untersuchungen an Pollenkörnern und Pollenschlaüchen von *Impatiens parviflora*. Ber. Deut. Bot. Ges. **52**: 43–47.

———. 1935. Ein Vergleich zwischen Kultur- und Griffelpräparaten von Pollenschläuchen von *Narthecium ossifragum*. Beih. Bot. Centralbl. **54A**: 83–98.

———. 1939*a*. Die Pollenentwicklung der Juncaceen. Jahrb. Wiss. Bot. **87**: 533–556.

———. 1939*b*. Die Entwicklung der Pollenkörner von *Triglochin palustris* L. und die verschiedenen Typen der Pollenkornentwicklung der Angiospermen. Jahrb. Wiss. Bot. **88**: 141–168.

——— and MAHESHWARI, P. 1938. The male gametophyte of angiosperms (a critical review). Jour. Indian Bot. Soc. **17**: 117–140.

WULFF, H. D. and RAGHAVAN, T. S. 1937. Beobachtungen an Pollenschlauchkulturen von der Hydrophyllaceae *Nemophila insignis*. Planta **27**: 466–473.

WUNDERLICH, R. 1936. Vergleichende Untersuchungen von Pollenkörnern einiger Liliaceen und Amaryllidaceen. Öst. Bot. Zeits. **85**: 30–55.

———. 1937. Zur vergleichenden Embryologie der Liliaceae-Scilloideae. Flora **32**: 48–90.

WYLIE, R. B. 1904. The morphology of *Elodea canadensis*. Bot. Gaz. **37**: 1–22.

———. 1923. Sperms of *Vallisneria spiralis*. Bot. Gaz. **75**: 191–202.

———. 1941. Some aspects of fertilization in *Vallisneria*. Am. Jour. Bot. **28**: 169–174.

YASUDA, S. 1929. Physiological researches on the fertility of *Petunia violacea*. VI. Growth of the pollen-tubes in the style. Bot. Mag. Tokyo **43**: 156–169.

————. 1930. Physiological researches. VIII. On the self-fertilizing ability of flowers in buds of self-incompatible plants. Bot. Mag. Tokyo **44**: 678–687.

ZIEGENSPECK, N. 1926. Über Amyloidfenster in den Narbenpapillen des *Alopecurus* und *Phleum*. Ber. Deut. Bot. Ges. **44**: 561–563.

TRIARCH BOTANICAL PRODUCTS

Fine Microscope Slides for Critical Botanists

* * * * *

Nothing can take the place of a good microscope slide in botany instruction, especially to show clearly that structure is a basis for function. However, the slide must be *good,* even though it is not perfect. If the slide can be beautiful as well as clear, it becomes a fine piece of teaching equipment.

TRIARCH slides, we believe, embody these points more completely than any other slides on the market, as we stain them regularly with our famous quadruple combination of safranin, crystal violet, fast green and gold orange, unexcelled for tissue differentiation. Also, as we stain all sections by the same schedule, our slides give you comparative checks on age and development of tissues. Many cytological points are emphasized by Triarch stain, and host-parasite relations are clearly demonstrated in pathological tissues.

Increasing numbers of botanists are realizing the teaching value of Triarch slides, together with the convenience of Triarch accuracy and prompt service, as evidenced by the fact that we are now shipping out more than 10,000 slides per month. Over 650 colleges and universities in 19 different countries are now counted among our customers.

If you are not already one of our patrons, send for our current catalog, No. 7, which lists over 350 regular catalog items, heavily stocked, together with many supplementary structures for special orders.

* * * * *

Geo. H. Conant

Triarch Botanical Products :-: Ripon, Wisconsin

Outstanding McGraw-Hill Books

AN INTRODUCTION TO BOTANY.

Second edition

By ARTHUR W. HAUPT, University of California at Los Angeles. *McGraw-Hill Publications in the Botanical Sciences.* 425 pages, $3.75

Covers the fundamental facts and principles involved in the structure, functions, life relations, and evolution of plants. The book does not attempt to present an exhaustive treatment of the subject, but to furnish a foundation upon which more advanced studies may be based.

ECONOMIC BOTANY.

A Textbook of Useful Plants and Plant Products

By ALBERT F. HILL, Harvard University. *McGraw-Hill Publications in the Botanical Sciences.* 592 pages, $5.00

Presents a clear, simple, and interesting discussion of the more important useful plants and plant products of the world from the standpoint of their history, cultivation, preparation, and utilization, with a view to emphasizing the influence which plants have had on history and civilization, and their importance in the everyday life of mankind.

DISEASES OF FIELD CROPS

By JAMES G. DICKSON, University of Wisconsin. *McGraw-Hill Publications in the Agricultural Sciences.* 401 pages, $4.50

Represents a complete revision and expansion of the author's privately printed *Outline of Diseases of Cereal and Forage Crop Plants of the Northern Part of the United States.* The present volume combines the pertinent historical and current information on the diseases of the more important crop plants.

A TEXTBOOK OF SYSTEMATIC BOTANY.

Third Edition

By the late DEANE B. SWINGLE, Montana State College. 343 pages, $3.50

This text embodies the suggestions of many of the best taxonomists in the country. The author meets the need for a textbook that approaches the subject by way of genetics, ecology, cytology, and geography.

Send for copies on approval

McGRAW-HILL BOOK COMPANY, Inc.

330 West 42nd Street **New York 18, N. Y.**

A NEW experience in laboratory microscopy

● You *feel* the difference . . . You *see* the difference . . . from the first moment you begin to focus a new Bausch & Lomb BAV Laboratory Microscope.

You obtain proper adjustment faster, more accurately, with less effort . . . superlative performance resulting from advanced mechanical features such as the variable focus condenser, threadless cell lens mounts, nose piece with roller bearing stop, coarse adjustment with *matched* rack and pinion, and patented lever fine adjustment.

Most important, you *see* better. Images of unmatched clarity and sharpness are provided by the world's finest optical system.

Before you buy a new laboratory microscope be sure to see the Bausch & Lomb BAV.

WRITE for a demonstration to Bausch & Lomb Optical Co., 708-A St. Paul St., Rochester 2, N. Y.

the Bausch & Lomb BAV Laboratory Microscope

THE BOTANICAL REVIEW

VOL. XV FEBRUARY, 1949 No. 2

WILD SPECIES–HYBRIDS IN THE PHANEROGAMS. II[1]

H. H. ALLAN

Dept. of Scientific and Industrial Research, Wellington, New Zealand

INTRODUCTION

When the previous article was published in 1937 it was necessary to argue that wild species-hybrids provide important material for taxonomic, genetic and evolutionary studies. The decade just past has shown a growing recognition of this, and important papers have appeared showing the value of combining studies of wild hybrids with work in the experiment garden. The upset of the war period has made it impossible, especially to one in an isolated country, to follow all the work in this field, and valuable contributions may have missed notice in this supplement. It is hoped, nevertheless, that the review will serve a useful purpose. The different fields of work have so interacted upon one another that it is no longer possible or desirable to confine discussion merely to field observations. The synopsis serves to show that there has been definite progress towards that desired rapprochment between workers in diverse fields.

FIELD OBSERVATIONS

These serve to provide indications of suitable subjects for more intensive studies. While no attempt is made here fully to cover the mass of observations the world over, it is hoped that a sufficiently representative selection has been given.

Field studies were published at various times in the decade on the following genera:

Agropyron x *Elymus* x *Hordeum* (196)
Agropyron x *Elymus* x *Sitanion* (195)
Agrostis (161), accepting the natural occurrence of *A. stolonifera* x *Polypogon monspeliensis.*
Alnus (56)
Ambrosia (172)
Anagallis (136, 154)
Aster (177)
Balsamorhiza (157)
Bromus (125), confirmed experimentally.
Carex (175)

---

[1] Supplement to article in The Botanical Review 3: 593–615. 1937.

Carmichaelia (179), no evidence of hybridism.
Ceanothus (141), degree of fertility of hybrids accepted as an index of relationship between the parental species.
Celmisia (138) ; (180), doubtful hybrid, apparently sterile, with *Olearia.*
Centaurea (163)
Centaurium (91)
Cephalanthera (160)
Chaenactis (199)
Coriaria (156), forming a complete linkage between the seven species recognized.
Cornus (26, 164, 165), yielding a diversity of fertile offspring.
Dactylorchis (197)
Danthonia (213)
Elymus × *Agropyron* × *Sitanion* (195)
Elymus × *Agropyron* × *Hordeum* (196)
Epilobium (198)
Epimedium (184), crossing with *Vancouveria* takes place in gardens but doubtfully in the wild.
Eucalyptus (28, 140)
Haastia (139)
Hevea (64)
Hordeum × *Agropyron* × *Elymus* (196)
Leucogenes (2), sterile hybrids with *Raoulia.*
Malus (142)
Mentha (119), regarding the Swedish forms of *M. gentilis* as "ein Schwarm von Abkömmlingen aus der Kreuzung *M. arvensis* × *spicata*".
Olearia (180), doubtful hybrid, apparently sterile, with *Celmisia.*
Oryzopsis × *Stipa* (120)
Oxalis (174), after prolonged search found no satisfactory evidence of natural hybridism in South African species, though opportunity by proximity is not infrequent.
Parthenium (169), 162 hybrids from immediate crosses in nature were identified from a greenhouse population of some 205,000 plants grown from seeds collected in the wild.
Pedicularis (171)
Poa (124)
Populus (172) ; (123), discusses controlled F_2 progeny derived from F_1 individuals arising from a natural cross between *Populus alba* and *P. grandidentata.*
Potamogeton (202)
Prunus (176), between an indigenous and a naturalized species.
Quercus (134)
Raoulia (2), sterile hybrids with *Leucogenes.*
Salix (71, 78)
Sitanion × *Agropyron* × *Elymus* (195)
Solidago (77, 95)
Sonchus (24)
Stipa (135) × *Oryzopsis* (120)
Vancouveria (184), crossing with *Epimedium* takes place in gardens but doubtfully in the wild.
Verbena (143)
Viola (198)

RELATION TO TAXONOMY

Whether the taxonomist has any concern with hybridization, either in the wild or in the experiment garden, has been debated. Bremekamp (26) argues that the taxonomist "is not interested in the origin, but in the characters of his plants". Allan (4) affirms that the study of wild hybrids is an essential aspect of taxonomic work and that purely observational work can yield information on material likely to be useful in more detailed and experimental studies. The widespread nature of wild hybridism in the flora of New Zealand, and the occurrence of multiform hybrid swarms is illustrated. Bremekamp (27) reiterates that for purposes of classification it is not the origin that counts but the character complex. Uittien (204) considers that for taxonomy hybrids do not exist, since taxonomy and its nomenclature are based on morphology, not on parentage. Fosberg (82, 83) vigorously combats the views of Bremekamp and Uittien. He maintains that genetics is one of the fundamental bases of the new experimental taxonomy, with systematic hybridization as its most powerful tool. He considers that one who "refuses to indicate hybridism is either over-cautious, or has never worked on a group of plants which produces hybrid swarms". Gilmour and Turrill (92) insist that the taxonomist must as far as possible consider all the attributes of living things, of which hybridism is one. There should be a proper taxonomic treatment of hybrids. Murbeck (146) agrees that hybridization must be considered in the delimitation of species, and gives instances in *Arctium, Celsia, Potentilla, Verbascum* and *Viola.* Palmer (159) says that "the plea that species should be defined on the basis of genetic relationship is commendable. That is what taxonomy attempts to do".

Arwidsson (16) lists for the region studied hybrids in *Betula, Cerastium, Draba, Juncus, Ranunculus, Rubus* and *Salix.* He also admits seven hybrids in *Carex* (40 species present) but points out that many supposed *Carex* hybrids are abnormal forms attacked by *Cintractia.* Camp (31–34) discusses hybrids in *Gaultheria* and *Pernettya* (and between the two genera), hybrid swarms in *Befaria,* and the nomenclatorial problems involved. "Such so-called 'species' are met with all too frequently in the Ericales in such genera as *Befaria, Rhododendron, Gaultheria, Pernettya,* and *Vaccinium*". In *Crataegus* he advises caution until the genetic structure is better known. In a useful discussion of "The herbarium in modern systematics" he remarks, "The day of the taxonomist who putters alone in his herbarium with an other-worldly stare is done. He must shed his robe of academic classicism and seclusion, brush off the accumulated dust of the centuries, and come face to face with the dynamics of living populations".

Chaytor (37) lists 23 hybrid forms in *Lavandula* and remarks, "that morphologically different plants may have had the same species as their immediate or original parents is to be explained by the intra-specific variation of several of the more widespread species and by the probable occurrence of segregation and back-crossing". Cugnac (57–59) continued his studies on *Bromus,* reproducing a form that "has been extinct for about 50 years". Fassett (68), in populations of *Linaria,* found internal barriers to hybridization and recognized only one doubtful hybrid. Fassett (71–75) also studied in some detail three species of *Juniperus,* including hybrid swarms. The examination of large collections of material showed that "Colonies in the east are pure *J. virginiana;* in Kansas occasional plants show a slight tendency toward *J. scopulorum;* in Nebraska this tendency is stronger; in the Bad Lands of South Dakota colonies show a completely scrambled mixture of characters of the two, then grade off to pure *J. scopulorum* in the west". The resultant complex is ascribed to recent and more ancient meetings. He recognizes two varieties of *J. virginiana* and two of *J. scopulorum,* but one in each species is perhaps a hybrid with *J. horizontalis.*

Griesinger and Klinkowski (106) record four spontaneous interspecific hybrids in *Ornithopus,* linking four of the five recognised species. Failure to produce hybrids experimentally is attributed to faulty technique. Howard and Manton (114) show that the wild triploid *Nasturtium officinale* is a hybrid between the wild diploid and tetraploid. The name *N. officinalc* is restricted to the diploid, and a new name *N. uniseriatum* is given to the wild allotetraploid. Johnson (120) found that the type specimens of *Oryzopsis bloomeri* and of *O. caduca* show that these "species" are hybrids between *O. hymenoides* and *Stipa occidcntalis* and *S. viridula,* respectively. Kramer (129), studying natural populations of guayule and mariola, found evidence of interspecific hybridization. Lewis and Epling (133) give field and experimental evidence that *Delphinium gypsophilum,* a constant diploid species, arose from the crossing of *D. recurvatum* and *D. hesperium.* Nannfeldt (147, 151) discusses a sterile hybrid between *Poa supina* and *P. annua* and its distribution.

Nygren (155) synthesized *Calamagrostis purpurea* both by colchicine treatment of *C. canescens* and by crossing *C. canescens* with *C. epigeios.* Apomixis is attributed to the combination of complementary genes in the hybrid zygote. Palmer (159) considers that hybrids undoubtedly occur in *Crataegus,* all species probably being potentially inter-fertile, but hybridization is greatly restricted by certain physiological and physical factors. Polunin (162) adopts a cautious attitude as regards supposed *Carex* hybrids, and a cautious attitude in general. Rollins (170) found that crosses linking five species of *Parthenium* were easily obtained. Stebbins

(190) gives evidence that *Bromus arizonicus* is an allopolyploid derived from the crossing of *B. henkeanus* and *B. trinii.* Stebbins and his co-workers (195, 196) describe artificial and natural hybrids in the Hordeae, the experimental supporting the field evidence. Intergeneric hybrids involving the linking of *Agropyron, Elymus, Hordeum,* and *Sitanion* are described. Nomenclatorial problems are discussed, and it is considered that the results indicate the need for a drastic revision of the taxonomy of the tribe.

Frandsen (86), crossing *Brassica campestris* and *B. nigra,* obtained amphidiploids. The data obtained supported the view that *B. juncea* may be an amphidiploid hybrid of natural origin. Stockwell and Richter (200) discuss the status of *Pinus attenuata* and *P. radiata* in view of the occurrence of fertile F_1 and F_2 hybrids. Camp (35) states that "no effective sterility barriers exist between the populations of *Cinchona* in the Andes of Ecuador"; where they now chance to meet, complete series of intergrades usually occur. Some have given rise to extensive secondary independently reproducing groups. There is an intergrading of both morphological and biochemical characteristics. Cleland (49) points out the difficulties that face the taxonomist dealing with *Oenothera,* and gives analyses of several "races" from the cytogenetic point of view. He considers that there is strong suggestion "that hybridization has played an important rôle in the production of the various races". Through natural crossing, diverse complexes have been brought together. Many of these hybrids have bred true, owing to the lethals present, and have become established as the true breeding races or species. We are confronted, therefore, with the possibility that a large percentage of the *Onagra* population is actually of hybrid origin.

Clausen and his co-workers (39–48) have made valuable contributions to "experimental taxonomy". Discussing the various degrees of incompatibility found in different species crosses, they conclude that a large percentage of the hybrids produced will be wiped out under natural conditions and that the few vigorous offspring left will largely drop back into one of the two original species. They agree that introgression may occur and that new amphidiploid species may arise. An amphidiploid origin through hybridization is postulated for a species of *Madia,* but amphidiploidy is considered to be a rare evolutionary path in the Madiinae. *Layia hieracioides* was experimentally proved to be an amphidiploid. They recognize that "as modern genetics developed in this century, it was rediscovered that hybridity is not a rare phenomenon and that many hybrids between species are partially fertile". Systematic hybridization is accepted as a "powerful analytical tool", while the use of the terms "ecotype", "ecospecies" and "coenospecies" is deprecated where experimental evidence is lacking. A vigorous but completely sterile *Madia* x *Layia* hybrid occurred in a culture from seed collected from a wild population. Two amphidiploids, one of *Madia,* one of *Layia,* occurred spontaneously in the experiment garden. Four species of *Potentilla* appeared to be linked by natural hybrids. The thesis: "taxonomic relationship between forms is roughly proportional to their ability to exchange genes either directly or indirectly through an intermediary", is illustrated by examples from *Artemisia, Pentstemon* and *Zauschneria.* Two natural hybrids in a *Layia* population "duplicated in appearance and behaviour the much larger population previously produced in the experiment garden". Crosses within a group of 14 species of *Layia* indicated a mosaic relationship as to "the relative ease or difficulty with which genes can be transmitted back and forth from one species to another". The data are assembled in an excellent diagram (45). Three amphiploids in the Madiinae, arising from sterile F_1 hybrids, without chemical or physical treatment, were thoroughly analysed and indicate what can happen in the wild. The initiation of amphiploidy within a mature comparium tends to regenerate it. The conclusion is reached that "Evolution may be said to be reticulate in pattern from the level of the ecotype to that of the comparium, but beyond that level it becomes exclusively forked in type". *Aquilegia* was found to combine extreme morphological and ecological variability with a high degree of crossability. In general, "Ecologically and taxonomically the amphiploid reacts as an interspecific F_1 hybrid that has become constant", and "successful amphiploids arise from successful interspecific hybrids . . . if the amphiploid is to remain successful during succeeding generations, the original balance must remain unchanged". It is considered that successful amphiploids will arise only from hybrids between species "so distantly related that their chromosomes cannot pair with each other or only rarely do so".

Gregor (104, 105), as a result of hybridization experiments, concludes "that, when given an opportunity to hybridize, the North American—North European—diploid Alpine population [comprising five recognised species of *Plantago*] constitutes a single intrafertile group, although in such a widespread population the potential gene exchange is not realized in nature owing to the spatial isolation of its parts". Taxonomic difficulties, arising as a result of gene exchange, lead Gregor to advocate that the experimental taxonomist should use a terminology based on the ecotype concept and not on the "present system of classification". He points out the rather frequent "misuse of the ecotypic terminology, particularly in botanical literature", and emphasizes the necessity that "The extensive, and in many respects promiscuous, collecting of museum material must perforce largely be replaced by an intensive, orderly and planned assembling of representative samples from the all-important breeding communities".

Experimental taxonomy, as it becomes developed and its principles clarified, seems destined to play an important part in that synthesis of results from varied lines of botanical research that to many botanists, and biologists in general, seems so necessary for real advance.

THE GENUS RUBUS

The genus *Rubus,* as treated by different workers, well illustrates the divergence of views on taxonomic principles and practice that still exists when genera of large size are being studied. Rydberg (173) recognised 112 species in the North American flora, 69 of which are not recorded as producing hybrids. For the remaining 43 species 90 hybrid groups are admitted (including three garden hybrids). Under this treatment we find a large comparium involving 28 species, in which *R. argutus* (12 crosses), *R. baileyanus* (10 crosses), *R. canadensis* (15 crosses), *R. nigrobaccus* (14 crosses), *R. procumbens* (12 crosses), play a large part in the linkage. Also the Arctici and Saxatiles are linked through *R. pubescens.* Rydberg by no means regarded all these hybrids as definitely established.

Bailey (22) gives a detailed discussion of the genus in North America. He recognises 390 species and a number of varieties, claiming that he has taken a broad view and has not split the species finely. Based on over 50 years of intensive study in the field, and a great mass of herbarium material, the monograph deserves close study. Especially useful are the notes on field work and methods of collecting and description. He admits no hybrids and in his detailed treatment of the species pays but slight attention to those holding other views. He remarks, "It is not to be understood that I am opposed to recognition of hybrids in *Rubus*. My objection is to the unscientific assumption that if a specimen, be it ever so poor, cannot be referred to any species at that moment recognized it must perforce be a hybrid. One may find in the field what are apparently wild hybrids, but they appear to be no more common or any more puzzling than in any other large genus". He rightly says, "Hybridity is not an assumption, but a subject capable of investigation". Of Rydberg's treatment he says, "It carried the hybridity assumption to its highest point in this country". Bailey considers that the conclusions of Rydberg (he is surely incorrect in stating that Rydberg admitted 153 hybrids in 36 species of *Eubatus*) and Brainerd (not seen by me) "disclose the prevailing incompetence in *Rubus* and remove the native blackberries from taxonomic study. Other genera than *Rubus* begin to show the break-away from former assignments of miscellaneous hybridity". "First", he says, "we must learn the species, that we may have starting-points; then we may speculate on hybridity and other questions". But surely, in such a group, the problem of what are the species and what are hybrids must be studied *pari passu,* and present dicta on species need investigation as much as dicta on hybrids. Not speculation but experimental study will yield results of value.

Crane and Darlington (55) had concluded that "The possibilities of crossing among species of *Rubus* are evidently determined to a great extent by the purely numerical relations of their chromosome complements. Hybrids are known to occur between the most distant species, yet crossing is often difficult between those having the greatest affinity". Hegi (112) said, "Die Gattung *Rubus* ist mit mindestens 300, bei engerer Fassung des Artbegriffes mit über 3000 Arten fast über die ganze Erde verbreitet. So schwer ihre Sektionen und Arten zu gliedern sind, so gut ist sie selbst abgegrenzt". He admits 19 major species within the area covered by his "Flora", but most of these are collective species and are split up into smaller species, with a total of 87. Hybridism is accepted as prevalent, but space was not available for any full treatment. It is suggested that a number of species are of hybridogenous origin.

Gustaffson (107–109) accepts the occurrence of hybrids in the Scandinavian *Rubi.* For most of the Eubati "there is a great variation due to hybridization", so that in the different groups there is a large number of transitional forms, while a great many localised and endemic microspecies have arisen. Even several "good" species are heterozygous to a "surprisingly great extent". Crosses between them or even segregation products may therefore give origin to "floras" of blackberries so rich in biotypes that each limited area contains its own endemism. Allopolyploidy is declared to be common, and the occurrence of apomicts has been substantiated. We now "know the principle outlines of the European blackberry flora, its systematics and geography". Lidforss, states Gustaffson, had proved that the Corylifolii are primary and secondary cross products of the Moriferi (Eubati) veri and the tetraploid *R. caesius.* "The astounding confusion of local and widely distributed types is simply due to the occurrence of pseudogamy, *i.e.,* parthenogenetic embryo formation accompanied by sexual endosperm development, maintaining in nature heterozygous segregates and hybrid derivatives". Further, "The apomicts in *Rubus* have not lost their capacity of sexual seed production but take part in a prosperous equilibrum system of apomixis and sexuality".

Judging from published descriptions and from the apparent difficulty in ascribing "varieties" to a particular species, the Asiatic *Rubi* would repay the like intensive and experimental study. In the New Zealand flora five species of *Rubus* are recognized, some with heteroblastic development, and all subject to modification by habitat conditions. Wild hybrids are very rare, and apparently sterile, though capable of vigorous vegetative growth. An artificial hybrid has occasionally produced flowers, but these show various abnormalities, and so far no viable seed has been produced.

NOMENCLATORIAL QUESTIONS

These have received some attention during the past decade, without any general agreement being reached. That the treatment in the International Rules is in need of modification is evident from the discussions aroused. A vigorous controversy was entered upon between Miss M. L. Green (99, 100) and Furtado (88, 89), arising from the problem of the correct name of the yellow hybrid aster. The two authors came to quite divergent views on the meaning and application of the Rules. Furtado comments that "there is no reason why systematic names of any sort of hybrids should receive a different treatment from the systematic names of non-hybrid taxonomic groups of corresponding rank", and suggests that "it should be recommended in the Rules not to give a systematic name to any hybrid, unless there are reasonable grounds for believing the hybrid to be stable or homozygous". Green considers "that there is every reason for according [the technical names of hybrids] different treatment". She points out the possibility of "confusion of thought between a genus and a generic name". Furtado makes a lengthy rejoinder and concludes that "the procedure adopted by Miss Green cannot be defended from the Rules of Botanical Nomenclature", and that if Miss Green's procedure "is the ideal one for naming hybrids it should be considered by the next Botanical Congress". Later Furtado agrees that "The Rules seem to be rather ambiguous on the status of the names of hybrids, and current practice is too contradictory to be of any use in expounding the correct application of the Rules". He gives several examples.

Allan (3) points out certain difficulties caused by the phenomenon of hybrid swarms. Numerous hybrid forms in the past have received specific names. Often what has been named represents only a small portion of a hybrid swarm. To extend the use of such names to cover all forms leads to confusion. "At present it is wisest to remain content with a formular treatment, and to treat the authority to provide names 'whenever it seems necessary or usual' as a privilege to be exercised with the utmost restraint". Sprague (183) considers that Allan has not distinguished between a group and its name. He suggests that names that are actually telescoped formulae should be restricted to hybrids whose origin has been proved experimentally.

Cain (29) points out that names of hybrids bestowed according to the International Code may be quite ambiguous in their application. He illustrates by reference to Oliver's method of naming hybrids in *Dracophyllum, e.g.,* ×*D. erectum* (*D. prostratum* ×*rosmarinifolium*). "The geneticist, ecologist, or geographer wonders what kinds of hybrids such names represent . . . one wonders how a taxonomist can draw up a description to cover a natural hybrid swarm and, if his description is based on one of a few hybrid forms, whether he realizes that he has entered on an almost endless descriptive task".

Uittien (204) considers that the words "hybrids" and "half-breeds" should be suppressed from the Rules. For the taxonomist, he considers, hybrids do not exist. But hybrids exist in nature, and it is difficult to see how the taxonomist can avoid considering them, unless, indeed, he does as Uittien suggests and relies purely on his eyes. The term "species" would then come to mean nothing more than a "described form". Fosberg (83) found no evidence that taxonomists in general incline to Uittien's views.

Stout (201) discusses the nomenclature of cultivated plants only, but his remarks contain matter of interest to the student of wild hybrids.

ANALYSIS OF POPULATIONS

Anderson (7) evolved a method of analysing hybrid populations in *Tradescantia* that, it is claimed, diagrammatically reveals their construction. The plants were scored by pairs of contrasting characters, each character being assigned a numerical value. The not altogether happy term "mass collection" was introduced by the same author (10, 11) for the critical sampling of entire populations. Anderson and Hubricht (12, 13), by analytical methods, studied "introgressive hybridization; the infiltration of the germplasm of one species into that of another by repeated back-crossing of the original hybrids". Only slight morphological effects were noted in the cases studied. Confirmatory hybrids were secured in the experiment garden. Riley (166–168), by similar methods, studied colonies of *Tradescantia* and *Iris*. In the *Tradescantia* colony a character analysis was made of 52 plants, the entire region being inhabited by three species. Few of the plants corresponded in every detail to any of the species. Crossing was common between the two tetraploid species, but uncommon between the diploid and the tetraploids. The triploid progeny were highly sterile. Evidence of introgressive hybridization in *Tradescantia* was also secured. A comparative study of seven characters on plants of four populations of *Iris* showed that one was pure *Iris hexagona* var. *giganticaerulea,* one pure *I. fulva.* Two colonies, situated geographically between the two pure species, in an area disturbed by man, showed the presence of hybrids that had become well established. "Many of the types considered species by Small show the same relation to the two species . . . that the hybrids of this study exhibit, and are probably themselves hybrids". These methods show a distinct advance, but do not gain complete objectivity; there is still room for unconscious bias in weighting the index values.

Anderson and Turrill (15) made detailed studies on two populations of *Fraxinus,* from collections representing random samples, one from the Danube delta, and one from the Mesta delta. The data suggest "that two species are involved, and that though much mixing has taken place since the two met they still tend to maintain their entities". The two hybrid populations agreed in their main features, but differed in the degree of approach to the two species concerned. Marsden-Jones and Turrill (137) analyzed samples of *Silene* populations from Norway and Switzerland, showing "peculiar combinations of characters which, it is suggested, may represent ancestral combinations from which *S. maritima* and more typical *S. vulgaris* have been derived". Charles and Goodwin (36) used analytical methods in establishing that the intergrades between two species of *Solidago* were natural hybrids. Larisey (132) analyzed a hybrid complex between two species of *Baptisia,* finding a considerable measure of poor pollen.

Fassett (66–70) studied by the mass collection method populations of *Rubus, Ranunculus, Diervillea, Lonicera, Oxalis* and *Linaria.* Analytical methods were also used in various papers cited elsewhere in this article.

GENETICAL AND CYTOLOGICAL STUDIES

Allan (1) showed the remarkable differences in fruit colour in the F_2 of a cross between two species of *Coprosma* (female grandparent blue; male, red) ranging from milk-white through yellow to scarlet, orange and red, with blue spotting more or less developed in many, but blue as the main colour in very few. A similar but not so extensive range of colour is seen in wild populations. Anderson (8) gave a review of cytology in relation to taxonomy, including the significance of amphidiploidy. Anderson (9), under the term "binary variation", shows that associated with typical *Tradescantia bracteata* throughout its range, and connected with it by a manifold series of intermediates, is a form of *Tradescantia* morphologically similar to *T. hirsutiflora.* This variant may have originated by partial differentiation from or hybridization with the

T. hirsutiflora-virginiana stock at a time when the ranges of these species were confluent. Anderson and Sax (14) deal with cytological aspects of the *Tradescantia* work and give further evidence on introgression.

Babcock and Stebbins (19) show that in *Crepis* many autopolyploids are as apomictic as allopolyploids. Baldwin (23) concludes that *Sedum pulchellum* is an amphidiploid that has undergone autopolyploidy. Certain resulting taxonomic confusions are pointed out. Charles and Goodwin (36) estimate that a minimum of 21 genes for leaf-characters, and twice as many for all morphologically distinguishable differences, is required to differentiate two species of *Solidago*. Clausen (38) studied polyploidy in *Nicotiana* and gives a résumé of the position. He considers that there is abundant evidence that *N. rustica* and *N. tabacum* are amphidiploids, and that the relationship to South American species is well established. Darrow and Camp (60) show that in *Vaccinium* and related groups both autopolyploidy and allopolyploidy have taken part in producing polyploid populations. A complex phyletic pattern has developed. Dillewijn (62) found strong cytological evidence for the existence of hybridism in *Populus*. Flovik (79), discussing the vexed question of the origin of hereditary vivipary in grasses, gives evidence that allopolyploidy is a cause in various species. The viviparous arctic grasses are allopolyploids, and vivipary is more common in arctic than in other grasses, owing to combined action of hybridization, polyploidy and extreme external conditions.

Fothergill (84, 85) examined wild populations of *Viola lutea* and *V. tricolor*, and made progeny tests. Many combinations of the parental characters occur, but plants not intermediate tend to resemble *V. lutea* more than *V. tricolor*. The hybrids showed increase of chromosome numbers beyond that expected from those of the parents, apparently owing to the splitting of univalents. Neither of the original species was recovered in its entirety. Frankel and Hair (87) found that *Hebe* has basic numbers 20 and 21, and includes tetraploid and hexaploid species. Interspecific crosses were completely successful only between 20-chromosome species; other species crosses gave rise to progeny of low vitality. Goodspeed (94) gives a full review of work on the cytotaxonomy of *Nicotiana*. He concludes that "The evidence of phylogenetic relationship in *Nicotiana* which is intrinsic in the morphological and distributional data appears to be sufficiently confirmed and amplified by the cytological conclusions to justify the current taxonomic arrangement of the genus".

Goodwin (95–98) confirmed the reputed hybridity of *Solidago asperula* by artificial hybridization. Hybrids and back-crosses occur in abundance where *S. rugosa* of open meadows is able to meet *S. sempervirens* of salt marshes. A morphological analysis of leaf-characters by Anderson's method (7) indicated that back-crosses and perhaps F_2 and other more complicated crosses frequently occur. In a study of seedling development it was found that the F_1 hybrids show a very strong matroclinous tendency. Greenleaf (101–103) found that cytological studies support the view that *Nicotiana tabacum* arose as an amphidiploid from *N. sylvestris* and some one of the several races of *N. tomentosum,* or *N. tomentosiformis.* This occurred in the general region of South America (Peru, Bolivia, northern Argentina) where these species and races occur to-day. Hair (110) found no irregularities in the meiosis of 17 species of *Epilobium* and three possibly hybrid forms. Heilborn (111) studied the chromosomes of Cyperaceae and concluded that allopolyploidy seems to be lacking in the family. B. L. Johnson (121), working on *Oryzopsis,* found that the section Piptatherum and the genus *Stipa* in part represent divergent lines of specialization and may have reticulated to form the polyploid section Eriocoma. With Rogler (122) he adduced strong cytological evidence for an *Oryzopsis* x *Stipa* hybrid. L. P. V. Johnson (123) noted some features of F_1 and F_2 hybrids in a *Populus* cross.

Kostoff (126–128), continuing his studies on polyploids in *Nicotiana,* synthesised a fertile amphidiploid by crossing *N. sylvestris* and *N. tomentosiformis.* He discussed numerous inter-specific crosses, several giving rise to amphidiploids. Müntzing (144) argues that "it is typical of species hybrids that they are more or less sterile", and that "by crossing experiments it is possible to clear up the barriers of incompatibility and sterility occurring in nature". He concludes that "If the cross succeeds without difficulty, and the hybrids obtained are quite fertile, the parents must be closely related and must be included in the same species". If this were followed by a New Zealand taxonomist the evidence at present available would strongly suggest that the manifold forms of *Coriaria* should again be united under one name, as was suggested by Lindsay in

1868 (Contributions to New Zealand Botany). In *Hebe* one would have largely to go back to the views of Mueller (The vegetation of the Chatham-Islands) who in 1864 lumped many species under his all embracing name of *Veronica forsteri.* Danser's method seems distinctly preferable for such cases.

Müntzing (145), continuing his studies on apomixis and sexuality in *Poa,* finds that "the evidence now available strongly suggests that the viviparous types are differentiation products, arisen by mutations from non-viviparous strains". He remarks, however, that "the possibility must not be overlooked that the whole species complex, *Poa alpina,* might be of hybrid origin". Nannfeldt (149, 153) gives evidence that the viviparous *Poa jemtlandica* is a hybrid and that the present population "is of rather high age, descending from a limited number of successful crossings, perhaps from a single one". Certain sterile hybrids in *Poa* show no trace of vivipary. Others, possessing viviparous biotypes, form both viviparous and non-viviparous hybrids. It is concluded from a survey of the evidence that "vivipary in all European and Arctic hybrids is explained by the hypothesis of vivipary as a hereditary dominant character. . . . No facts support the view that vivipary is induced in hybrids as a compensation for their floral sterility". The section Stoloniferae of *Poa* is thought probably to have arisen from intersectional hybridization. It contains no uniform species; the constant units are non-sexual subspecies.

Östergren (158) studied the cytology of *Agropyron junceum, A. repens* and their wild hybrids. Seven hybrids were found to be pentaploid and one heptaploid by back-crossing of a pentaploid with *A. junceum,* or from the fertilisation of an unreduced gamete of *A. junceum* with a normal one from *A. repens.* Silow (178) brings out evidence in support of Skovsted's view that the New World tetraploid cultivated cottons arose by amphidiploidy following hybridization between ancestral Asiatic and American diploid species. Smith (181) gives evidence from various sources that long continued isolation does not necessarily result in cross-incompatibility, nor does great morphological divergence. Stebbins (186), after a review of the data concerning apomixis in the angiosperms, finds that the great majority of apomictic forms are probably allopolyploids or back-cross derivatives from allopolyploids. The hybridity is an accompanying phenomenon but not a causal agent of apomixis. Stebbins (189) reviewed also work on the cytological analysis of species hybrids. The evidence is considered to support the hypothesis that "speciation comes about either through hybridization between pre-existing species or through superposition of genetic isolating factors upon a pattern of intraspecific differentiation into races or subspecies, as postulated by Mayr". Stebbins and his co-workers (192–194) found that all but a few of the *Crepis* apomicts are allopolyploids, that *Bromus carinatus* and its relatives form a complex of polyploids (many being allopolyploids), and that *B. arizonicus* is an allopolyploid derived from *B. catharticus,* or an ancestral form, and *B. trinii.* Wetmore and Delisle (209) confirmed that *Aster amethystina* is a natural hybrid. *A. multiflorus* and *A. novae-angliae* hybridize extensively in nature, with back-crossing, producing a polymorphic group, to forms of which have been given different names by taxonomists. Wheeler (210), from cytological examination of 15 species of *Nicotiana* found in Australia and the Southern Pacific, and of the meiotic behaviour of numerous hybrids, suggests that amphidiploidy has been important in the evolution of the group, together with selfing or back-crossing after former hybridization.

ECOLOGICAL SIGNIFICANCE

As early as 1923 Cockayne (50), discussing hybridism in the New Zealand flora, remarked: "It is the fact of considerable hybridization taking place in a *virgin* vegetation which is the leading motive for this paper. But the *effect of settlement* is also an important matter. . . . In these man-induced associations a few species have found more opportunities for crossing than formerly so that their hybrids are now far more numerous than in primeval New Zealand. To the powerful effect of man in unconsciously favouring hybridisation Professor Aug. Henry has recently called my attention". Instances are given in the genera *Acaena, Cassinia, Celmisia* and *Fuchsia.* A particular *Nothofagus* forest had been replaced by a subalpine vegetation in which *Celmisia* hybrids were prominent at a much lower altitude than their normal habitats. Cockayne, Simpson and Thompson (53), describing new communities that had arisen following the destruction of the primitive vegetation by man, referred to the occurrence of hybrid groups in *Acaena, Aristotelia, Cassinia, Celmisia, Coriaria, Gaultheria, Hebe, Helichrysum* and *Myrtus*. Cockayne and Sledge (52) mention several hybrids as being present in a particular area where there had developed a herbfield replacing burnt forest.

Weigand (211), studying occurrences of *Amelanchier, Crataegus* and *Rubus,* found that an outburst of crossing may occur locally in areas in which the primitive vegetation has been disturbed, *e.g.,* by forest fires, lumbering or clearing of railroad tracks. One result of the disturbance may be irregularities in the time of flowering. "The hybrids seem like swarms of bees, buzzing around for a time, only to disappear, leaving the fundamental species to continue through the ages". He discusses genetic reasons for this disappearance and considers that taxonomists tend to collect undue proportions of unusual forms. The importance of the disturbance of natural vegetation and the production of intermediate habitats is also stressed for *Aster* (11), *Tradescantia* (12), *Solidago* (36, 96) and *Iris* and *Tradescantia* (167, 168).

Hubricht and Anderson (115) state that certain species of *Tradescantia* readily hybridize by natural agencies in the experiment garden, but that in nature, owing to lack of intermediate habitats, hybridism is rare. Hybridization is more active along the Appalachians, as more intermediate zones are found there than elsewhere. Darrow and Camp (61) adduce evidence that the present prevailing hybridism in *Vaccinium* has been made possible by "the disturbance of the ecological balance—the extensive and repeated clearing and burning, the formation of fields and pastures, and often their later abandonment". Bees are considered to be the agents of pollination. "In the blueberry there seems to be no interspecific sterility between homoploids". Each species seems to have a basic genom set which is compatible with that in other species. Because self-sterility occurs to a greater or lesser extent in the genus, the development of species seems to have been determined more by geographical and ecological isolation than by genetic isolation. Smith (182) states that artificial hybrids have been produced in abundance in *Populus,* and the comparative scarcity of natural hybrids is attributed to the different ecological habitats favoured by the different species. Stebbins (187) discusses the general question of the rôle of isolation in the differentiation of plant species. Turrill (203) points out that *Alopecurus geniculatus* and *A. pratensis* will hybridize but are kept "essentially distinct" by their different habitatal preferences. Several similar cases are referred to. He concludes, "There is, however, no doubt that man is likely to become increasingly important in modifying nature by enabling new species to establish themselves. Thus by cutting down forests on the hill-slopes in the Nearer East species of *Verbascum, Thymus, Dianthus, Silene,* and other genera have been able to extend their areas, to meet, and to hybridize. That certain segregates or allopolyploids resulting from such hybridization may be selected or are in process of selection seems probable". Heine (113) thought that "The similarity and simplicity in the structure of the flowers and the great extent to which cross-pollination is brought about by a variety of insects may be regarded as one of the causes of the large number of wild hybrids in New Zealand". Cain (29) points out that "Such centers of variation as are due to hybridization and polyploidy may develop at the center of origin of a genus, but that is not necessarily the case". The same author (30), in a general treatment of the principles of plant geography, gives a great deal of space to the influence of wild hybridism, with a wide range of illustrative material, whereas Wulff (212), treating plant geography from the historical point of view, finds little occasion to refer to wild hybridism.

EVOLUTIONARY SIGNIFICANCE

The importance and limitation of polyploidy, more especially allopolyploidy, as a factor in evolution have been increasingly explored, with considerable reference to wild allopolyploids. The phenomena are discussed in some detail in most recent standard texts (*e.g.,* 30, 63, 93, 117). Huxley (117) sums up, "Allopolyploidy has undoubtedly played an important rôle in the evolution of many plant genera". Allopolyploidy "is likely to occur when changes of climate bring about range-changes, these then providing opportunities for hybridisation between plant species which have developed in isolation and between which no reproductive barriers have therefore been evolved". Huxley also discusses the bearing of wild hybridism in general on the evolution of plants, and its relation to apomixis and "reticulate evolution". Where "some of the products of initial hybridization continue to cross, we obtain elaborate complexes such as those of *Rosa, Rubus, etc.,* in which a number of initial forms are combined in an evolutionary reticulum".

Stebbins (185) agrees that polyploidy tends to break down genetic barriers and to permit exchange of genes between genetic systems that in the diploid condition are completely isolated. He gives examples to show that where autopolyploidy and allopolyploidy exist in the same genus hybridization, especially when associated with apomixis, may result in a "complex network of interrelated forms, which defies classification according to the usual concepts of the species". Warmke (207) suggests that polyploidy may offer a means of securing new genes required in the building of plant and animal kingdoms. Babcock and his co-workers (17–19, 21) discuss genetic evolutionary processes in *Crepis*. Gene mutations and structural changes in the chromosomes are primary, and interspecific hybridization, polyploidy and apomixis are secondary processes. The main trend has been a progressive decrease in chromosome numbers, but in special groups there has been an increase due to hybridization followed by amphidiploidy.

Barber (25) finds that the two species of *Paeonia* in California and Oregon are "true-breeding diploid hybrids of the kind known in *Oenothera*". Babcock *et al.* (20) find that the American species of *Lactuca* are amphidiploids that have probably crowded out the ancestral diploids. Erlanson (65) considers that in *Rosa* the hybridization of hexaploids with related diploids, followed by the elimination of unpaired chromosomes in the descendants, is "an important *modus operandi* in promoting the appearance of polymorphic diploid races with novel characteristics". Lamprecht (131) studied the nature of the interspecific barriers in a *Phaseolus* cross, where in the sterile hybrid mitosis was normal, but meiosis did not proceed beyond tetrad formation. Some evolutionary significance is ascribed to the types of barriers discussed.

Ford (80, 81) distinguishes transient polymorphism, in which a gene is spreading through a population, from balanced, in which the gene is maintained at a fixed level by opposed selective agencies. Under certain conditions the free flow of genes is impeded, evolutionary change reduces the stock of genes, "leading to infertility on crossing and to specific distinction".

PRACTICAL APPLICATIONS

Following the demonstration by Allan and Zotov (5) that *Phormium colensoi* could be crossed with *P. tenax*, supporting the view that the polymorphy noted in nature is due in part to hybridism, Swindlehurst (unpublished) raised a large F_1 progeny, the plants combining in some degree the strength of the one species with the softness of fibre of the other. Tests have shown that the cross offers distinct possibilities in the textile industry. Crane (54) gives examples of wild hybrids that have proved useful in the production of forms of horticultural value. Darrow and Camp (61) show how natural *Vaccinium* hybrids may be used in securing new material of economic value. Wild forms of *Leptospermum* in New Zealand have been introduced into gardens on account of specially attractive features of flower colour and "doubling". Lammerts (130) successfully crossed certain horticultural "varieties" and secured strains of enhanced merit. He discusses the genetic problems raised.

Love (135) points out that natural interspecific hybridization in *Stipa* may be utilized in developing useful strains. Stebbins (190) points out the practical significance of hybridization in *Bromus*. Weibel and Woodworth (208) discuss the use of the "natural-crossing plot" in making castor-bean hybrids. In general, it is clear that there is a wide field for profitable investigation on the practical application of the data arrived at in the study of wild hybrids.

POSTSCRIPT

Baker (Nature **159**: 221–223), under the title "Criteria of hybridity", deplores the "decline" of experiment since Kerner's day. He gives a useful criticism of the methods of Anderson and others, using the index method of scoring hybrids, in that they too greatly depend upon the observer's powers of discrimination. He says that "It is easy to overcome the faults in these methods by making it a rule that, wherever possible, artificial crosses between the forms involved (giving evidence of the mode of inheritance of the characters) should *precede* investigations of natural populations". He realises some of the difficulties in carrying out such work and goes on to consider how the most suitable characters can be selected and used in analytical work.

There has of course been experimental work on the lines Baker advocates, but it can hardly be denied that much more work should be done. The results of his own work will be awaited with interest. Baker makes the encouraging, if bold, statement that "The importance of the study of natural hybridization is obvious in view of the large part it plays in the process of evolution".

LITERATURE CITED

1. Allan, H. H. Fruit characters in the F_2 of a *Coprosma* cross. Trans. Royal Soc. New Zealand **68**: 34–36. 1938.
2. ————. Remarks on ×*Leucoraoulia*. Trans. Royal Soc. New Zealand **68**: 457–461. 1939.
3. ————. The nomenclature of hybrids. Chron. Bot. **5**: 205–209. 1939.
4. ————. Natural hybridization in relation to taxonomy. New Systematics, Oxford 1940: 515–528.
5. ———— and V. D. Zotov. An artificial cross between *Phormium colensoi* and *P. tenax*. New Zealand Jour. Sci. & Tech. **18**: 799–804. 1937.
6. Anderson, E. The species problem in *Iris*. Ann. Mo. Bot. Gard. **23**: 457–509. 1936.
7. ————. Hybridization in American tradescantias. I. A method for measuring species of hybrids. II. Hybridization between *T. virginiana* and *I. canaliculata*. Ann. Mo. Bot. Gard. **23**: 511–525. 1936.
8. ————. Cytology in its relation to taxonomy. Bot. Rev. **3**: 335–350. 1937.
9. ————. Binary variation in *Tradescantia bracteata*. Ann. Mo. Bot. Gard. **28**: 147–163. 1941.
10. ————. The technique and use of mass collections in plant taxonomy. Ann. Mo. Bot. Gard. **28**: 287–292. 1941.
11. ————. Mass collections. Chron. Bot. **7**: 378–380. 1943.
12. ———— and L. Hubricht. Hybridization in *Tradescantia*. III. The evidence of introgressive hybridization. Am. Jour. Bot. **25**: 396–402. 1938.
13. ———— and ————. The American sugar maples. I. Phylogenetic relationships as deduced from a study of leaf variation. Bot. Gaz. **100**: 312–323. 1938.
14. ———— and K. Sax. A cytological monograph of the American species of *Tradescantia*. Bot. Gaz. **97**: 433–476. 1936.
15. ———— and W. B. Turrill. Statistical studies on two populations of *Fraxinus*. New Phyt. **37**: 160–172. 1938.
16. Arwidsson, T. Studien über die Gefässpflanzen in den Hochgebirgen der Pite Lappmark. Acta Phyt. Suecica XVII. 1943.
17. Babcock, E. B. and M. S. Cave. A study of intra- and interspecific relations of *Crepis foetida* L. Zeits. Ind. Abst. Ver. **75**: 124–160. 1938.
18. ———— and J. A. Jenkins. Chromosomes and phylogeny in *Crepis*. III. The relationships of one hundred and thirteen species. Univ. Calif., Pub. Bot. **18**: 241–292. 1943.
19. ———— and G. L. Stebbins. The American species of *Crepis*. Carnegie Inst. Wash., Pub. 504: 1–199. 1938.
20. Babcock, E. B. *et al.* Chromosomes and phylogeny in some genera of Crepidinae. Cytologia. Fujii Jubilee Vol. 188–210. 1937.
21. ———— *et al.* Genetic evolutionary processes in *Crepis*. Am. Nat. **76**: 337–363. 1942.
22. Bailey, L. H. Species Batorum Boreali-Americana. Gentes Herbarum 5(1–10): 1941–1945.
23. Baldwin, J. T. Polyploidy in *Sedum pulchellum*. I. Cytogeography. Bull. Torrey Bot. Club **70**: 26–33. 1943.
24. Barber, H. N. Spontaneous hybrids between *Sonchus asper* and *S. oleraceus*. Ann. Bot. **5**: 375–577. 1941.

25. ———. Evolution in the genus *Paeonia*. Nature **148**: 227. 1941.
26. Bremekamp, C. E. B. Phylogenetic interpretations and genetic concepts in taxonomy. Chron. Bot. **5**: 390. 1939.
27. ———. Controversial questions in taxonomy. Chron. Bot. **7**: 255–258. 1942.
28. Brett, R. G. A survey of *Eucalyptus* species in Tasmania. Pap. & Proc. Royal Soc. Tasmania **1937**: 75–109.
29. Cain, S. A. Criteria for the indication of center of origin in plant geographical studies. Torreya **43**: 132–154. 1943.
30. ———. Foundations of plant geography. 1944.
31. Camp, W. H. Studies in the Ericales. IV. Notes on *Chimaphila*, *Gaultheria* and *Pernettya* in Mexico and adjacent regions. Bull. Torrey Bot. Club **66**: 7–28. 1939.
32. ———. Studies in the Ericales. A discussion of the genus *Befaria* in North America. Bull. Torrey Bot. Club **68**: 100–111. 1941.
33. ———. The *Crataegus* problem. Castanea **7**: 51–55. 1942.
34. ———. The herbarium in modern systematics. Am. Nat. **77**: 322–344. 1943.
35. ———. Biochemical clines, polymorphic populations, and the problems of specific delimitation in the cinchona population of Ecuador. In Am. Jour. Bot. **33**: 234. 1946. [Abs.]
36. Charles, D. R. and R. H. Goodwin. An estimate of the minimum number of genes differentiating two species of goldenrod with respect to their morphological characters. Am. Nat. **77**: 53–69. 1943.
37. Chaytor, D. A. A taxonomic study of the genus *Lavandula*. Jour. Linn. Soc. (Bot.) **51**: 153–204. 1937.
38. Clausen, R. E. Polyploidy in *Nicotiana*. Am. Nat. **75**: 291–306. 1941.
39. Clausen, J. *et al.* Experimental taxonomy. Carnegie Inst. Wash., Yearbook **35**: 208–214. 1936.
40. ——— *et al.*, Experimental taxonomy. Carnegie Inst. Wash., Yearbook **36**: 209–214. 1937.
41. ——— *et al.* The experimental taxonomist's terminology. Chron. Bot. **5**: 224–225. 1939.
42. ——— *et al.* The concept of species based on experiment. Am. Jour. Bot. **26**: 103–106. 1939.
43. ——— *et al.* Experimental taxonomy. Carnegie Inst. Wash., Yearbook **39**: 156–163. 1940.
44. ——— *et al.* Experimental studies on the nature of species. I. Carnegie Inst. Wash., Pub. 520: 451 pp. 1940.
45. ——— *et al.* Experimental taxonomy. Carnegie Inst. Wash., Yearbook **40**: 160–170. 1941.
46. ——— *et al.* Experimental taxonomy. Carnegie Inst. Wash., Yearbook **42**: 91–100. 1943.
47. ——— *et al.* Experimental taxonomy. Carnegie Inst. Wash., Yearbook **43**: 69–81. 1944.
48. ——— *et al.* Experimental studies on the nature of species. II. Plant evolution through amphiploidy and autoploidy, with examples from the Madiinae. Carnegie Inst. Wash., Pub. 564. 1945.
49. Cleland, R. E. Analysis of wild American races of *Oenothera* (*Onagra*). Genetics **25**: 636–644. 1940.
50. Cockayne, L. Hybridism in the New Zealand Flora. New Phyt. **22**: 105–127. 1923.
51. ———. On the occurrence of subalpine vegetation at a low level in the Fiord Botanical District (N. Z.) and of matters pertaining thereto. Flora **1925**: 75–80.
52. ——— and W. A. Sledge. A study of the changes following the removal of subalpine forest in the vicinity of Arthur's Pass, Southern Alps, N. Z. Jour. Linn. Soc. (Bot.) **49**: 115–131. 1932.

53. ———— *et al.* Some New Zealand indigenous-induced weeds and indigenous-induced modified and mixed plant-communities. Jour. Linn. Soc. (Bot.) **49**: 13–45. 1932.
54. Crane, M. B. The origin and behaviour of cultivated plants. New Systematics, Oxford, 1940. pp. 529–547.
55. ———— and C. D. Darlington. The origin of new forms in *Rubus*. I. Genetica **9**: 241–278. 1927.
56. Cretziou, P. Hibridul *Alnus glutinosa* × *Alnus incana*. Rev. Pădurilov **52**: 654–656. 1940.
57. Cugnac, A. de. Recherche phylètique sur le genre *Bromus*. VIII. Bull. Soc. Bot. France **86**: 409–419. 1939.
58. ————. Vers la solution expérimentale des problèmes phylètiques par l'étude des croissements interspecifiques. Compt. Rend. Acad. Sci. **211**: 143–146. 1940.
59. ————. Sur la transformation expérimentale d'une espèce monotype en espèce polymorphe. Compt. Rend. Soc. Bot. France **138**: 845–847. 1944.
60. Darrow, G. W. *et al.* Chromosome numbers in *Vaccinium* and related groups. Bull. Torrey Bot. Club **71**: 498–506. 1944.
61. ———— and W. H. Camp. *Vaccinium* hybrids and the development of new horticultural material. Bull. Torrey Bot. Club **72**: 1–21. 1945.
62. Dillewijn, C. van. Zytologische Studien in der Gattung *Populus* L. Genetica **23**: 131–182. 1940.
63. Dobzhansky, T. Genetics and the origin of species. 1941.
64. Ducke, A. Novas contribuicões para o contecimento das seringueras (Hevea) da amazônia brasiliera. Arq. Serv. Flor. Brasil **2**: 25–43. 1943.
65. Erlanson, E. W. Phylogeny and polyploidy in *Rosa*. New Phyt. **37**: 72–81. 1938.
66. Fassett, N. C. Mass collection of *Rubus odoratus* and *R. parviflorus*. Ann. Mo. Bot. Gard. **28**: 299–374. 1941.
67. ————. Mass collections: *Ranunculus abortivus* and its close relations. Am. Mid. Nat. **27**: 512–527. 1942.
68. ————. Populations of *Linaria* on the Gulf Coast. Am. Jour. Bot. **29**: 351–352. 1942.
69. ————. Mass collections: *Diervillea, Lonicera*. Bull. Torrey Bot. Club **69**: 317–322. 1942.
70. ————. Mass collections: *Oxalis violacea*. Castanea **7**: 31–38. 1942.
71. ————. *Juniperus virginiana, J. horizontalis* and *J. scopulorum*—I. The specific characters. Bull. Torrey Bot. Club **71**: 410–418. 1944.
72. ————. *Juniperus virginiana, J. horizontalis* and *J. scopulorum*—II. Hybrid swarms of *J. virginiana* and *J. scopulorum*. Bull. Torrey Bot. Club **71**: 475–483. 1944.
73. ————. *J. virginiana, J. horizontalis* and *J. scopulorum*—III. Possible hybridization of *J. horizontalis* and *J. scopulorum*. Bull. Torrey Bot. Club **72**: 42–46. 1945.
74. ————. *J. virginiana, J. horizontalis* and *J. scopulorum*—IV. Hybrid swarms of *J. virginiana* and *J. horizontalis*. Bull. Torrey Bot. Club **72**: 379–384. 1945.
75. ————. *J. virginiana, J. horizontalis* and *J. scopulorum*—V. Taxonomic treatment. Bull. Torrey Bot. Club **72**: 480–482. 1945.
76. Fernald, M. L. A hybrid cornus from Cape Breton. Rhodora **43**: 411–412. 1941.
77. ————. Technical studies in North American plants. Contr. Gray Herb. Harv. Univ. 160. 1946.

78. Floderus, B. The continental *Salix glauca* species. Svensk. Bot. Tidskr. **37**: 169–172. 1943.

79. Flovik, K. Cytological studies of arctic grasses. Hereditus **24**: 265–376. 1938.

80. Ford, E. B. Polymorphism and taxonomy. New Systematics, Oxford, 1940: pp. 4930513.

81. ————. Polymorphism. Biol. Rev. **20**: 73–87. 1945.

82. Fosberg, F. R. Taxonomy and hybridism. Chron. Bot. **5**: 397–398. 1939.

83. ————. For an open-minded taxonomy. Chron. Bot. **6**: 368. 1941.

84. Fothergill, P. G. Studies in *Viola*. I: The cytology of a naturally-occurring population of hybrids between *Viola tricolor* L. and *Viola lutea* Huds. Genetica **20**: 159–186. 1938.

85. ————. Studies in *Viola*. III: An account of the inheritance of certain characters in the progeny of a wild population of *Viola* hybrids. New Phyt. **40**: 139–151. 1941.

86. Frandsen, K. T. The experimental formation of *Brassica juncea* Czern. et Coss. Dansk. bot. Arkiv **11**: 1–7. 1943.

87. Frankel, O. H. and J. B. Hair. Studies in the cytology, genetics and taxonomy of New Zealand *Hebe* and *Veronica*. New Zealand Jour. Sci. & Tech. **18**: 669–687. 1937.

88. Furtado, C. X. The nomenclature of hybrids and of garden plants. Chron. Bot. **4**: 32–36. 1938.

89. ————. The nomenclature of hybrids. Fedde, Rep. **44**: 244–255. 1938.

90. ————. Amendments proposed to the international rules of botanical nomenclature (1935). Gard. Bull. S. S. **11**: 1–30. 1939.

91. Gilmour, J. S. L. Notes on the genus *Centaurium*. Kew Bull. **1937**: 497–502.

92. ———— and W. B. Turrill. The aim and scope of taxonomy. Chron. Bot. **6**: 217–219. 1941.

93. Goldschmidt, R. The material basis of evolution. 1940.

94. Goodspeed, T. H. Cytotaxonomy of *Nicotiana*. Bot. Rev. **11**: 533–592. 1945.

95. Goodwin, R. H. Notes on the distribution and hybrid origin of × *Solidago asperula*. Rhodora **39**: 22–28. 1937.

96. ————. The cytogenetics of two species of *Solidago* and its bearing on their polymorphy in nature. Am. Jour. Bot. **24**: 425–432. 1937.

97. ————. Studies on the seedling development of *Solidago rugosa* Mill., *S. sempervirens* L., and the reciprocal hybrids between them. Am. Jour. Bot. **24**: 627–640. 1937.

98. ————. Estimates of the minimum numbers of genes differentiating several taxonomic categories in the genus Solidago. Bull. Torrey Bot. Club **72**: 235–245. 1945.

99. Green, M. L. The correct name of the yellow hybrid Aster. Kew Bull. **1937**: 350–352.

100. ————. On the validity of the name *Solidaster*. Chron. Bot. **4**: 36–39. 1938.

101. Greenleaf, W. H. Induction of polyploidy in *Nicotiana*. Jour. Hered. **29**: 451–464. 1938.

102. ————. Sterile and fertile amphidiploids. Their possible relation to the origin of *Nicotiana tabacum*. Genetics **26**: 301–324. 1941.

103. ————. Genic sterility in *Tabacum*-like amphidiploids of *Nicotiana*. Jour. Genet. **43**: 69–96. 1942.

104. Gregor, J. W. Experimental taxonomy. IV. Population differentiation in North America and European sea-plantains allied to *Plantago maritima* L. New Phyt. **38**: 293–322. 1939.

105. ————. The units of experimental taxonomy. Chron. Bot. **7**: 193–196. 1942.

106. Griesinger, R. and M. Klinkowski. Geographie und Cytologie des europäischen Formenkreise der Gattung *Ornithopus*. Züchter **11**: 147–161. 1939.
107. Gustaffson, Å. Skandinaviens Rubus flora. Bot. Not. **1938**: 378–420.
108. ————. Differential polyploidy within the blackberries. Hereditas **25**: 33–47. 1939.
109. ————. The origin and properties of the European blackberry flora. Hereditas **28**: 249–277. 1942.
110. Hair, J. B. The chromosome complements of some New Zealand Plants. Trans. Royal Soc. New Zealand **71**: 271–276. 1942.
111. Heilborn, O. Chromosome studies in Cyperaceae. III–IV. Hereditas **25**: 224–240. 1939.
112. Hegi, G. Illustrierta Flora Mittle Europas Bd. IV/2: 759–805.
113. Heine, E. M. Observations on the pollination of New Zealand flowering plants. Trans. Royal Soc. New Zealand **67**: 133–148. 1937.
114. Howard, H. W. and I. Manson. Autopolyploid and allopolyploid watercress, with the description of a new species. Ann. Bot. **10**: 1–13. 1946.
115. Hubricht, L. and E. Anderson. Vicinism in *Tradescantia*. Am. Jour. Bot. **28**: 957. 1941.
116. Huxley, J. S. The new systematics. 1940.
117. ————. Evolution. The modern synthesis. 1942.
118. ————. Species and evolution. Endeavour **5**: 3–12. 1946.
119. Hylander, N. De svenska formerna av *Mentha gentilis* L. coll. Acta Phyt. Suecica **14**: 1–49. 1941.
120. Johnson, B. L. Natural hybrids between *Oryzopsis hymenoides* and several species of *Stipa*. Am. Jour. Bot. **32**: 599–608. 1945.
121. ————. Cytotaxonomic studies in *Oryzopsis*. Bot. Gaz. **107**: 1–32. 1945.
122. ———— and Rogler, G. A. A cyto-taxonomic study of an intergeneric hybrid between *Oryzopsis hymenoides* and *Stipa viridula*. Am. Jour. Bot. **30**: 49–56. 1943.
123. Johnson, L. P. V. A note on inheritance in F_1 and F_2 hybrids of *Populus alba* L. × *P. grandidentata* Michx. Am. Jour. Bot. **24**: 313–317. 1946.
124. Kiellander, C. L. En ny Poa-hybrid, *Poa palustris* × *Poa compressa*. Bot. Not. **1944**: 363–372.
125. Knowles, P. F. Interspecific hybridization of *Bromus*. Genetics **29**: 128–140. 1944.
126. Kostoff, D. Studies on polyploid plants, XVIII. Cytogenetic studies on *Nicotiana sylvestris* × *N. tomentosiformis* hybrids and amphidiploids and their bearings on the problem of the origin of *N. tabacum*. Compt. Rend. Acad. Sci. U.R.S.S. **18**: 459–462. 1938.
127. ————. Studies on polyploid plants. XXI. Cytogenetic behaviour of the allopolyploid hybrids *Nicotiana glauca* Grah. × *Nicotiana langsdorfii* Weinm. and their evolutionary significance. Jour. Genet. **37**: 129–208. 1938.
128. ————. Cytogenetics of the genus *Nicotiana*. [Abs. in Pl. Breed. Abs. **16**: 374. 1946].
129. Kramer, H. H. The evaluation of individual plant selections from a natural population of guayule, *Parthenium argentatum* Gray. Jour. Am. Soc. Agron. **38**: 22–31. 1946.
130. Lammerts, W. E. New double flowering *Leptospermum* hybrids. Jour. Cal. Hort. Soc. **6**: 250–257. 1945.
131. Lamprecht, H. Die genisch-plasmatische Grundlage der Art-barriere. [Abs. in Pl. Breed. Abs. **16**: 302. 1946].
132. Larisey, M. M. Analysis of a hybrid complex between *Baptisia leucantha* and *Baptisia viridis* in Texas. Am. Jour. Bot. **27**: 624–628. 1940.

133. Lewis, H. and C. Epling. Formation of a diploid species of *Delphinium* by hybridization. Am. Jour. Bot. **33**: 235–236. 1946. [Abs.]

134. Little, E. L. New names in *Quercus* and *Osmanthus*. Jour. Wash. Acad. Sci. **33**: 8–11. 1943.

135. Love, R. M. Interspecific hybridization in *Stipa* L. Am. Nat. **80**: 189–182. 1946.

136. Marsden-Jones, E. M. and F. E. Weiss. The essential differences between *Anagallis arvensis* Linn. and *Anagallis foemina* Mill. Proc. Linn. Soc. London, Sess. **150**: 146–155. 1938.

137. ———— and W. B. Turrill. Researches on *Silene maritima* and *S. vulgaris*. XXIV. Kew Bull. **1939**: 465–473.

138. Martin, W. Species of *Celmisia* indigenous to Marlborough, and descriptions of new species and varieties. Trans. Royal Soc. New Zealand **65**: 169–186. 1935.

139. ————. Notes on the indigenous flora of Marlborough (New Zealand) with special reference to plant distribution. Trans. Royal Soc. New Zealand **67**: 414–425. 1938.

140. McAulay, A. L. Evidence for the existence of a natural hybrid between *Eucalyptus globulus* and *E. ovata*. Pap. & Proc. Royal Soc. Tasmania **1937**: 45–46.

141. McMinn, H. E. The importance of field hybrids in determining species in the genus *Ceanothus*. Proc. Cal. Acad. Sci. **25**: 323–356. 1944.

142. McVaugh, R. The status of certain anomalous native crab-apples in eastern United States. Bull. Torrey Bot. Club **70**: 418–429. 1943.

143. Moldenke, H. N. The known geographic distribution of the members of the Verbenaceae and Avicenniaceae. Supp. 2. Bot. Gaz. **106**: 158–164. 1944.

144. Muntzing, A. Genetics in relation to general biology. Hereditas **24**: 492–504. 1938.

145. ————. Further studies on apomixis and sexuality in *Poa*. Hereditas **26**: 115–190. 1940.

146. Murbeck, S. Bastarder och artsystematik. Bot. Not. **1943**: 314–332.

147. Nannfeldt, J. A. *Poa supina* Schrad. i Sverige och dess hittills förbisedda hybrid med *Poa annua* L. Bot. Not. **1935**: 1–16.

148. ————. Taxonomical and plant-geographical studies in the *Poa laxa* group. Symb. Bot. Upsalla 1(5). 1935.

149. ————. On *Poa jemtlandica* (Almqu.) Richt., its distribution and possible origin. A criticism of the theory of hybridization as the cause of vivipary. Bot. Not. **1937**: 1–27.

150. ————. The chromosome numbers of *Poa* sect. Ochlopoa and their taxonomic significance. Bot. Not. **1937**: 238–254.

151. ————. Om *Poa supinas* utbredning i Norden. Bot. Not. **1937**: 258–265.

152. ————. *Poa maroccana* Nannf. n. sp. and *P. rivulorum* Maire and Trabut, two more tetraploids of sect. Ochlopoa A. & Gr., and some additional notes on *Ochlopoa*. Svensk. Bot. Tidstkr **32**: 295–321. 1938.

153. ————. On the polymorphy of *Poa arctica* with special reference to its Scandinavian forms. Symb. Bot. Upsalla 4(4). 1940.

154. Nilsson, H. *Anagallis arvensis* L. s. l. und die Natur ihrer Farben varianten. Hereditas **24**: 97–109. 1938.

155. Nygren, A. The genesis of some Scandinavian species of *Calamagrostis*. Hereditas **32**: 131–262. 1946.

156. Oliver, W. R. B. The genus *Coriaria* in New Zealand. Rec. Dom. Mus., Wellington **1**: 21–43. 1942.

157. Ownbey, M. and W. A. Weber. Natural hybridization in the genus *Balsamorhiza*. Am. Jour. Bot. **30**: 179–187. 1943.

158. Östergren, G. Cytology of *Agropyron junceum, A. repens* and their spontaneous hybrids. Hereditas **26**: 305–316. 1940.

159. Palmer, E. J. The species concept in *Crataegus.* Chron. Bot. **7**: 373–375. 1943.

160. Petterson, B. *Cephalanthera longifolia* (L.) Fritsch. × *C. rubra* Rich. på Gotska sandön. Bot. Not. **1941**: 114–122.

161. Philipson, W. R. A revision of the British species of the genus *Agrostis.* Jour. Linn. Soc. (Bot.) **51**: 73–150. 1937.

162. Polunin, N. Contributions to the flora and phytogeography of south western Greenland: an enumeration of the vascular plants with critical notes. Jour. Linn. Soc. (Bot.) **52**: 349–406. 1943.

163. Quer, P. F. Una nueva *Centaurea hibrida.* Bol. Soc. Brot. **19**: 261–263. 1944.

164. Rickett, H. W. *Cornus stolonifera* and *Cornus occidentalis.* Brittonia **5**: 149–159. 1944.

165. ————. Cornales. No. Am. Flora **28B**: 297–316. 1945.

166. Riley, H. P. Hybridization in a colony of *Tradescantia.* [Abs. in Genetics **22**: 206–207. 1939].

167. ————. A character analysis of colonies of *Iris fulva, Iris hexagona* var. *giganticaerulea* and natural hybrids. Am. Jour. Bot. **25**: 727–737. 1938.

168. ————. Introgressive hybridization in a natural population of *Tradescantia.* Genetics **24**: 753–769. 1939.

169. Rollins, R. C. Evidence for natural hybridity between guayule (*Parthenium argentatum*) and mariola (*Parthenium incanum*). Am. Jour. Bot. **31**: 93–98. 1944.

170. ————. Interspecific hybridization in *Parthenium.* Pt. II. Am. Jour. Bot. **33**: 21–30. 1946.

171. Ronniger, R. Neuheiten aus der Flora von Kärntens. Fedde Rep. **48**: 268–270. 1940.

172. Rouleau, E. Notes taxonomiques sur la flore phanérogamique du Québec. Nat. Canad. **71**: 265–272. 1944.

173. Rydberg, P. A. *Rubus.* No. Am. Flora **22**: 428–480. 1913.

174. Salter, T. M. The genus *Oxalis* in South Africa. Jour. So. Afr. Bot. **1**(Suppl.). 1944.

175. Saxén, U. Die varietaten von *Carex salina* Wg. ssp. *suspidata* Wg. nebst ihren Hybriden an den Küsten des Bottnischen Busens, Finnland. Acta Bot. Fenn. **22/23**: 1–30. 1938.

176. Sernander, R. *Prunus spinosa* × *Prunus insititia* i den svenska växtvärlden, jämte några ord om *Prunus-arternas* växtgeografiska ställning i densamma. Acta Phyt. Suecica **13**: 101–200. 1940.

177. Shinners, L. H. The genus *Aster* in Wisconsin. Am. Mid. Nat. **26**: 398–420. 1941.

178. Silow, R. A. Evidence on chromosome homology and gene homology in the amphidiploid New World cottons. Jour. Genet. **47**: 213–221. 1946.

179. Simpson, G. A revision of the genus *Carmichaelia.* Trans. Royal Soc. New Zealand **75**: 231–287. 1945.

180. ———— and J. Scott Thomson. Notes on some New Zealand plants and descriptions of new species (2). Trans. Royal Soc. New Zealand **72**: 21–40. 1942.

181. Smith, E. C. Chromosome behavior in *Catalpa hybrida* Spaeth. Jour. Arn. Arb. **22**: 219–221. 1941.

182. ————. A study of cytology and speciation in the genus *Populus* L. Jour. Arn. Arb. **24**: 275–305. 1943.

183. Sprague, T. A. The nomenclature of hybrids. Chron. Bot. **5**: 209–212. 1939.

184. STEARN, W. T. *Epimedium* and *Vancouveria* (Berberidaceae), a monograph. Jour. Linn. Soc. (Bot.) **51**: 409–534. 1938.
185. STEBBINS, G. L. The significance of polyploidy in plant evolution. Am. Nat. **74**: 54–60. 1940.
186. ——————. Apomixis in the angiosperms. Bot. Rev. **7**: 507–542. 1941.
187. ——————. The role of isolation in the differentiation of plant species. Biol. Symp. **6**: 217–233. 1942.
188. ——————. The concept of genetic homogeneity as an explanation of the existence and behavior of rare and endemic species. Chron. Bot. **7**: 252–253. 1942.
189. ——————. The cytological analyses of species hybrids 11. Bot. Rev. **11**: 463–486. 1945.
190. ——————. Artificial synthesis of old and new polyploid species in *Bromus*. [Abs. in Genetics **32**: 107–108. 1947].
191. —————— and E. B. BABCOCK. The effect of polyploidy and apomixis in the evolution of species in *Crepis*. Jour. Hered. **30**: 519–530. 1939.
192. —————— and J. A. JENKINS. Aposporic development in the North American species of *Crepis*. Genetica **21**: 191–224. 1939.
193. —————— and H. A. TOBGY. The cytogenetics of hybrids in *Bromus*. I. Hybrids within the section Ceratochloa. Am. Jour. Bot. **31**: 1–11. 1944.
194. ——————, —————— and J. R. HARLAN. The cytogenetics of hybrids in *Bromus*. II. *Bromus carinatus* and *Bromus arizonicus*. Proc. Cal. Acad. Sci. **25**: 307–322. 1944.
195. —————— *et al.* Artificial and natural hybrids in the Gramineae, tribe Hordeae. I. *Elymus, Sitanion,* and *Agropyron*. Am. Jour. Bot. **33**: 338–351. 1946.
196. —————— *et al.* Artificial and natural hybrids in the Gramineae, Tribe Hordeae. II. *Agropyron, Elymus* and *Hordeum*. Am. Jour. Bot. **33**: 579–586. 1946.
197. STEPHENSON, T. A new hybrid *Dactylorchis*. Jour. Bot. **80**: 104. 1943.
198. STERNER, R. Flora der Insul Öland. Acta Phyt. Suecica **9**: 169 pp. 1938.
199. STOCKWELL, P. A revision of the genus *Chaenactis*. Contr. Dudley Herb. Stanford Univ. **3**: 89–167. 1940.
200. —————— and F. I. RICHTER. *Pinus:* the fertile species hybrid between knobcone and Monterey pine. Madroño **8**: 157–160. 1946.
201. STOUT, A. B. The nomenclature of cultivated plants. Am. Jour. Bot. **27**: 339–347. 1940.
202. TAYLOR, J. M. and W. A. SLEDGE. ×*Potamogeton cognatus* Asch. and Graeb. in Britain. Naturalist, October-December, 1944: 121–123.
203. TURRILL, W. B. Ecological isolation. Kew Bull. **1938**: 384–390.
204. UITTIEN, H. Reflections on the nomenclature of so-called hybrids. Chron. Bot. **5**: 212–214. 1939.
205. WARBURG, E. F. Taxonomy and relationship in the Geraniales in the light of their cytology. Part I. New Phyt. **37**: 130–159. 1938.
206. ——————. Taxonomy and relationship in the Geraniales in the light of their cytology. Part II. New Phyt. **37**: 189–210. 1938.
207. WARMKE, H. E. Polyploidy and evolution. Am. Nat. **75**: 344–346. 1941.
208. WEIBEL, R. O. and C. M. WOODWORTH. Use of natural crossing plot in making castor bean hybrids. Jour. Am. Soc. Agron. **38**: 563–565. 1945.
209. WETMORE, R. H. and A. L. DELISLE. Studies in the genetics and cytology of two species in the genus *Aster*, and their polymorphy in nature. Am. Jour. Bot. **26**: 1–11. 1939.

210. Wheeler, H. M. A contribution to the cytology of the Australian-South Pacific species of *Nicotiana*. Proc. Nat. Acad. Sci. **31**: 177–185. 1945.
211. Wiegand, K. M. A taxonomist's experience with hybrids in the wild. Science **81**: 161–166. 1935.
212. Wulff, E. V. An introduction to historical plant geography. 1943.
213. Zotov, V. D. Certain changes in the nomenclature of New Zealand species of Gramineae. Trans. Royal Soc. New Zealand **73**: 233–238. 1943.

CYTOLOGY OF BACTERIA. II[1]

GEORGES KNAYSI
Laboratory of Bacteriology, State College of Agriculture,
Cornell University, Ithaca, N. Y.

INTRODUCTION

Since the author wrote (1938), in this journal, his first review on the cytology of bacteria, interest in the structure of the bacterial cell and knowledge of that structure have considerably increased. This was due to a number of reasons: development of the electron microscope; application of new techniques and refinement in old ones; development of new points of view. It would be presumptuous to say that present knowledge of the structure of the bacterial cell is complete. It is certain, however, that the progress of the last decade transformed bacterial cytology from a philosophy into a science in which structure and behavior are beginning to show relationships and common grounds. This, indeed, is the "raison d'être" of cytology.

Books and reviews published during the last ten years were the following: Imšenecki (1940), Lewis (1941), Knaysi (1944), Mudd and Anderson (1944), and Dubos (1945). This brief review will be devoted, chiefly, to the progress made during this period. The literature is covered till the closing months of 1947.

FORM AND SIZE OF THE BACTERIAL CELL

Form

There are three fundamental forms which can be assumed by a normal bacterial cell: the coccus form in which the cell is ellipsoidal or spherical; the rod form in which the cell is cylindrical or resembles a long ellipsoid; and the spiral form in which the cell is helicoidal. The terms "spherical", "ellipsoidal", "cylindrical" or "helicoidal" are descriptive of what approaches the corresponding geometrical form.

In a young or a mature culture growing under uniform conditions, a given form may predominate, but almost always deviation from that form may be observed. In a culture of *Streptococcus*, for instance, rod-like forms may often be observed; a culture of a rod-like strain may contain both coccus and helicoidal cells; cultures of *Vibrio* or *Spirillum* may contain coccus and rod-like cells. In old cultures the mixture of forms becomes more common, and many cells assume irregular or involution forms. Certain strains, called "pleomorphic", regularly exhibit mixtures of forms, even in young cultures. Variation of form in a uniform environment may be due to irregularities in cell division, as in a mixture of coccus and rod-like forms, or to local irregularities in the cell wall, as in a mixture of rod-like, wavy and helicoidal forms. In cells grown on the surface of agar, the shape of a cell may be altered by friction or other physical factors. Recent observations by Pijper (1946, 1947*a*) on *Eberthella typhosa* show that motility induces a helicoidal form which persists after motility ceases. Pijper (1946) states that "the conventional description of bacteria as rods is due to their appearance in killed, fixed and stained preparations. . .". Since the normal shape should be that characteristic of the active state, "the normal shape of a motile bacterium is not rod-like but is that of a spiral or a helix. To how many bacterial species this conception applies is difficult to say at the moment. It does not seem excluded that even those that are non-motile nowadays still preserve an indication of a previous more active period and shape." This view is opposite to that previously expressed by Ellis (1923) who considered *Spirillum* as a rod-like organism with a delicate membrane, which assumes a helicodial form because of its motion.

---

[1] Supplement to article in The Botanical Review 4: 83–112. 1938.

The form of a cell determines the relation between its surface area and its volume, and thus affects its relation to environment. The most valuable expression of form is the one which relates most closely the form of the cell to its volume and its surface area. In deriving such an expression, strictly geometrical form is assumed which in many instances is not too far from the truth. Expressions suitable for coccus and helicoidal cells were applied and discussed by Knaysi (1941*b*, 1944). No similar expression has yet been derived for rod-like cells, and the ratio between width and length is still used for this purpose. The mathematical study of form and its value may be illustrated by the work of Henrici (1928) and Knaysi (1941*b*).

Size and Other Dimensional Relations

Knaysi (1944) published formulae for the rectification of helicoidal and wavy dimensions as well as for the estimation of volumes and surface areas of cells for all three standard forms.

Experimental Determination of Form and Size

Almost always the form and size of the bacterial cell are determined directly by means of the microscope. The direct methods commonly used may be classified as follows:

1. With the electron microscope.
2. With the light microscope:
   - *a*) on living cells
   - *b*) on stained cells
   - *c*) on dried, "negatively stained" cells.

These various methods will now be discussed and evaluated.

With the Electron Microscope. Dubin and Sharp (1944) observed that "The appearance of *Bacillus megatherium* in electron micrographs is different from that of the same organism seen in light micrographs. Images in the electron micrographs indicate plainly the presence of two structures constituting the bacterial cell, an inner dense substance and an outer less dense substance. This outer substance of the bacterial cell is invisible in the light micrographs. . . . There is no significant difference between measurements of the size of unstained bacteria dried in air and photographed with the light microscope and measurements of the comparable portion (inner dense substance) of the identical bacteria later photographed in the electron microscope." A glance at the illustrations published by Dubin and Sharp shows that the "outer substance" which is invisible with the light microscope is the cell wall which surrounds the "inner dense substance of shrunken protoplasm."

Recent work by Hillier and Knaysi (1947*b*) showed that when a bacterial cell is dried in the air on a collodion film it becomes flattened and its protoplasm shrinks to a variable degree; the cell wall also shrinks but usually much less than the protoplasm. Collapse of the cell wall tends to increase the width of the cell in relation to its length and thus affects its form. However, this latter effect is more than counterbalanced by the shrinkage of the cell wall, so that cell width in electron micrographs is smaller than in the living cell.

With the Light Microscope: a) *On the living cell.* When a bacterial cell is observed in the living state, one observes only the protoplasm surrounded by the cytoplasmic membrane. The cell wall is not visible. Consequently, the cell appears slightly more slender and smaller than it is. Because of the thinness of the cell wall, however, the form and size observed by this method are nearest to the true form and size of the cell. This statement is true only when the cell is observed in its own culture medium. Transfer of the cell into a medium of different osmotic concentrations usually results in swelling or shrinkage of the cell. Dutky (1933) observed a linear cytoplasmic contraction of 14% to 25% when young cells of *Bacillus megatherium* were transferred from ordinary broth to broth containing NaCl in 2-molar concentration, and Lasseur, Dupaix, and Georges (1932) observed an increase in volume from 0.100 to 0.140 μ^3 when cells of *Pseudomonas caryocyanea* were transferred from physiological salt solution to distilled water.

b) On the stained cell. Until recently form and size were studied in dry, stained smears, but it was shown by Knaysi (1941*a*) that what one sees in such smears is a shrunken protoplasm which has neither the form nor the size of the living cell. On the other hand, when the bacteria are stained in a way that renders the cell wall visible, and observation is made on wet smears, the form and size of a cell are not significantly different from its form and size in the living state.

c) On dried, "negatively stained" cells. This has been a very popular method with certain investigators. Dubin and Sharp (1944) found that "Measurements of the size of *B. megatherium* obtained by Benians' Congo red method were less than those of the total bacterial substance seen in the electron micrographs." Knaysi (1944) pointed out that "Dry negative preparations are often unsuitable for the study of form, particularly in capsulated organisms, because the slime layer is included in the apparent outline of the cell." Capsulated organisms also appear considerably larger. Using *Bacillus mycoides,* Knaysi (1945*b*) showed that in the absence of capsules "the cells may appear larger or smaller than their real size depending on the thickness of the dye film surrounding the cells."

Variability of Form and Size

Since the bacterial cell is in direct contact with the environment, its form, size, metabolic activities and other characteristics are greatly affected by the environment. These aspects were adequately discussed by Knaysi (1944) who also pointed out that, at least in bacteria, what is usually attributed to age is the result of the process of growth or of alteration in the environment brought about by the activities of the bacteria themselves. Changes due to age were called "cytomorphosis" by Henrici (1928).

a. Imposed Environment. It was shown by Knaysi (1943*b*) that the effect of $MgSO_4$ on cell morphology, previously observed by Stapp and Zycha (1931) in *Bacillus mycoides*, may also be observed in other bacteria. In *Escherchia coli* $MgSO_4$ causes enlargement and deformation of the cells. Besides yeast-like cells, one observes ring-like forms and numerous enlarged curved cells often with pointed ends. The cytoplasm is highly vacuolated. This effect was correlated with osmotic pressure and permeability. Bayne-Jones and Sandholzer (1933) observed enlargement or swelling of the cells of *Escherchia coli* before lysis with the bacteriophage. Some of the enlargement was an expression of increase in cell substance, and was not altogether due to imbibition of water. This was not observed in *Bacillus megatherium.*

Most of the literature which appeared on this subject during the last ten years dealt with the effect of sulfa drugs (Gay and Clark, 1937; Lockwood, 1938; Tunnicliff, 1939), of penicillin (Gardner, 1940; Fleming, 1941; Smith and Hay, 1942; Hobby, Meyer, and Chaffee, 1942; Weiss, 1943; Miller and Foster, 1944; Miller, Scott and Moeller, 1944; Hobby and Dawson, 1944; Lee, Foley, and Epstein, 1944; Rantz and Kirby, 1944; Todd, 1945; Chain and Duthie, 1945; Thomas and Levine, 1945; Alture-Werber, Lipscitz, Kashdan, and Rosenblatt, 1945; Fisher, 1946; Shanahan, Eisenstark, and Tanner, 1947; Pratt and Dufrenoy, 1947; Dufrenoy and Pratt, 1947) and of tyrothricin (Johnson, 1944). The effect is expressed by enlargement and deformation of the cells, lysis and, in Gram-positive bacteria, often an alteration in this reaction. It is interesting that in pneumococcus the capsule persists after the protoplasm is lysed. The effect was found maximum during the active growth of the culture and was attributed by most investigators to interference with the mechanism of cell division. On the other hand, Garrod (1945) found the maximum effect at 42° C. after growth had stopped.

b. Age. Variation of form and size in a given medium during the early stages of development are largely due to growth and multiplication. The work of Clark and Ruehl (1919) and of Henrici (1928) on this subject was previously reviewed (Knaysi, 1938, 1944).

A cinematographic study of the growth of cell volume with time during active growth was made by Bayne-Jones and Adolph (1932*a*, 1932*b*) on the yeast *Saccharomyces cerevisiae* and on *Escherichia coli,* and by Adolph and Bayne-Jones (1932) on *Bacillus megatherium.* When the volume of a single yeast cell is plotted against time, an S-like curve is obtained; plotting the logarithms of the volume against time gives a curve which is continuously concave toward the time axis. In the case of *E. coli,* logarithms of the volume plotted against time give a wavy curve which, as the culture becomes older, tends to become irregular but seems to approach an S-shape. With *Bacillus megatherium* volume against time gives a curve continuously concave toward the volume axis, and the logarithms of the volume against time give a straight line. Adult lengths, breadths and volumes of the bacteria "were progressively modified at various ages of the culture. Mean sizes and total bulk in the culture appeared to depend upon rates of growth and of reproduction. Evidence was adduced that rates of growth controlled rates of reproduction. Adult sizes could be considered more primary than rates of reproduction in controlling the characteristics of individuals." Perhaps the most interesting aspect of this work is the finding that growth of the yeast cell ceases and resumes with the formation of each new bud, a relation not found between consecutive generations of the bacteria they investigated. This work was followed up by Knaysi (1940) who found that the rythmic relation in growth is also present in the dividing yeast *Schizosaccharomyces pombe* but not in *Bacillus cereus.* Consequently, rythmic growth is not the result of multiplication by budding. In a later study Knaysi (1941*b*) observed rythmic growth in *Streptococcus faecalis* and suggested that it may be a characteristic of uninuclear cells. The "resting" cell of this organism has the form of a slightly flattened ellipsoid; when transferred to a fresh medium it gradually elongates into a sphere and then into an elongated ellipsoid; when the volume reaches a maximum of 2.7–3.4 μ^3 the cell divides into two cells each having the shape of a flattened ellipsoid (*E* about –0.5). The width of the cell undergoes only a very slight change during the process. In *Bacillus cereus* there is no definite volume at which the cell divides. Knaysi also found that the cell of *Streptococcus faecalis* divides when the ratio of surface area to volume reaches a certain value (3.6–3.8). In *Bacillus cereus* this ratio is practically constant. When the entire culture of *Streptococcus faecalis* is considered, the predominating form and size are determined by the statistical condition of the culture with respect to form and dimensions of its cells, which is determined by the statistical conditions of the cells with respect to growth and multiplication. In young cultures the average cell volume fluctuates considerably with time and becomes more steady as the culture matures. The average form follows a somewhat parallel curve.

Dissociation

Inheritable changes in form and size may be caused by dissociation. It must be emphasized, however, that the resulting strains are affected by age and environment in the manner described above.

The literature on variations of this type is extensive and was recently reviewed by Braun (1947) and by Luria (1947). A characteristic tendency of this period has been to consider these changes chiefly mutational, a point of view which is justified by the similarity of organization between the cell of bacteria and that of other organisms. It is hoped, however, that the geneticist will show discrimination in applying knowledge gained from the study of higher organisms to bacteria.

STRUCTURE OF THE BACTERIAL CELL

During the last decade considerable progress was made in knowledge of the structural make up of the bacterial cell. The existence of a cytoplasmic membrane and a cell wall was definitely proved. A nucleus was demonstrated in a few species, and there are reasons to expect that its existence is more general than heretofore believed. The structure of the endospore was considerably clarified. The flagella became again a subject for investigation. Furthermore, much was learned about some important cellular processes, such as multiplication and spore formation. Notable advances were made in knowledge of the chemical and antigenic composition of various structures, and in certain instances their behavior was related to certain cell properties and cellular processes.

Cytoplasm and Cytoplasmic Membrane

The cytoplasm is that ground material which, together with the surrounding cytoplasmic membrane, occupies the cavity of the cell wall. When free of inclusions the cytoplasm is optically homogeneous and appears so even with the electron microscope. It is probably a colloidal system containing water, protein, lipoids, usually ribonucleic acid and other substances in which the relation to one another is uncertain. In *Spirillum volutans* the viscosity of the cytoplasm is not unusually high as compared to that of organisms other than bacteria (King and Beams, 1942).

The cytoplasmic membrane is a highly refractive and stainable structure of variable thickness. In the living cells of *Bacillus cereus* it varies in thickness from 0.2 to 0.3 μ (Knaysi, 1946a). In electron micrographs the cytoplasmic membrane of *Bacillus mycoides* appears very thin. The difference is probably due not only to a difference between organisms and to desiccation, but also to the fact that the thickness observed with the light microscope is magnified by a diffraction halo.

The cytoplasmic membrane normally stains with dyes of the Sudan series, gives the Sharp test for proteins, and a positive Feulgen reaction. These characteristics survive autolysis of the cytoplasm and treatment with fat solvents; it has an isoelectric range below that of simple proteins, even in Gram-negative species (Knaysi, 1946*a*). These reactions lead to the conclusion that the principal component of the cytoplasmic membrane is a stable combination of lipoids and proteins, namely, lipoprotein. There is also evidence that this lipoprotein molecule may be conjugated with some complex organic radical related to ribonucleic acid. It should be emphasized, however, that the composition of the cytoplasmic membrane is by no means constant; its lipoid and nucleic acid content varies considerably. The work of Knaysi and Baker (1947*b*) with the electron microscope showed that the membrane of *Bacillus mycoides* consists of molecules 40 Å to 60 Å in width in the form of beaded threads.

In the life of the cell the cytoplasmic membrane is extremely important in cell permeability and cell division. In *Bacillus cereus* and *Bacillus mycoides* it is a site of lipoid synthesis (Knaysi, 1945*a*, 1946*a*).

The Nucleus

During the last ten-year period a number of contributions were made to the study of the nuclear problem. Delaporte (1939–40) concluded and extended her early work (1934, 1935, 1936*a*, and 1936*b*) on the structure of bacteria and the Cyanophyceae. Using a number of staining procedures and the Feulgen reaction, she reported that in the cocci, chromatin is in the form of a granule; in rod-like bacteria, it is a granular rod which may break up into granules; in coccobacilli, such as *Azotobacter agilis*, it may be as in the cocci or as in rod-like cells; in *Spirillum* and the filamentous sulfur and iron bacteria, it is in the form of a central rod as in rod-like bacteria. Some of the red sulfur bacteria contain a central body; in *Chromatium* and *Achromatium* chromatin is either in the form of a central mass or fragmented into several portions. The paper includes an exhaustive review of the literature.

Stille (1937) reported that Feulgen-positive bodies may be readily observed in various bacteria if the temperature of hydrolysis is reduced to 40° C. Piekarski (1938, 1939*a*, 1939*b*, 1940) investigated the nucleus, using staining procedures, the Feulgen reaction and the ultra-violet and electron microscopes (see also Piekarski and Ruska, 1939). The results by all methods were similar: rod-like bacteria contain one or more nucleus-like bodies or nucleoids which divide before cell division; *Sarcina* contains a single central nucleoid. Young cells stained uniformly with acetic carmin or Giemsa's solution, but the nucleoids became readily demonstrable when the cells were stained after treatment with hydrochloric acid. Piekarski attributed the beneficial effect of HCl to loosening the structure of the cell membrane. Nucleoids were demonstrable with the electron microscope in individual cells from most old cultures of *Eberthella typhosa, Escherichia coli, Serratia marcescens, Proteus* and *Pseudomonas aeruginosa,* but rarely in cells of young cultures. In old cultures the cells contain a single central nucleoid; in young cultures there is a nucleoid at each end. Piekarski and Ruska (1939) confirmed these observations but found little structural differentiation in the cells of *Sarcina alba, Sarcina lutea* and *Bacillus subtilis.* Piekarski (1939*b*) was unable to observe structure in the nucleoid and concluded that it is not made up of individual elements (chromosomes). Chance (1938) and Allen, Appleby and Wolf (1939) observed nucleus-like bodies in members of *Bacillus.* Chance saw evidence of mitosis, and Allen *et al.* that of meiosis.

Knaysi (1941*c*, 1942) demonstrated in the cells of *Staphylococcus flavo-cyaneus* bodies which stained with methylene blue at pH 1.8–2.0, were insoluble in boiling water within ten minutes or in 0.02% $NaHCO_3$ within two hours, gave the Millon and the Sharp tests for protein and a positive Feulgen reaction. When the smears were placed in a solution consisting of 90 ml. of distilled water and ten ml. of conc. HCl for 24 hrs. at room temperature, the bodies were not demonstrable with methylene blue at pH 2.4, but were still demonstrable at pH 4.6 to 4.8. They were distinct from any inclusions known to occur in the bacterial cell. Resting cells contained a single granule, round or slightly ellipsoidal; actively growing cells contained from two to four granules of shapes indicating elongation and division. Knaysi concluded that these bodies were, both chemically and morphologically, of nuclear nature.

Robinow (1942, 1944, 1945) combined staining with Giemsa's solution after fixation of the cell suspension with vapors of osmium tetroxide (Dobell, 1911) and hydrolysis by HCl (Piekarski, 1939*a*). He was thus able to demonstrate in vegetative cells or endospores of a number of bacteria bodies which in young cultures showed a possible evidence of division. These bodies were called "chromatinic structures" or, following Badian (1933, 1935), "chromosomes." Lewis (1942) showed that the important step in the process is that of hydrolysis; in cells treated with HCl these bodies may be clearly seen after staining with methylene blue. According to Robinow (1945) the effect of hydrolysis is chiefly to reduce stainability of the cytoplasm in vegetative cells and to increase permeability of the membrane in the endospores. Robinow was unable to observe the same bodies with the electron microscope.

Knaysi and Mudd (1943) studied the internal structure of a number of bacteria with the RCA electron microscope. Organisms opaque to the electrons at 60 kilovolts were also observed at higher voltages. At 60 kv, the young cells of *Staphylococcus flavo-cyaneus*, *Staphylococcus aureus* and *Streptococcus pyogenes* appeared homogeneously opaque to the electrons; those of *Neisseria gonorrhoeae* were homogeneous and semitransparent; those of *Brucella abortus* and *Pasteuralla pestis* showed a homogeneous transparent protoplasm containing one to several bright vacuoles. Old cells of the latter two organisms showed a transparent protoplasm containing one or two opaque bodies of unknown nature. Mature cells of *Neisseria meningitidis* had a transparent protoplasm containing one opaque body per cell; this body was insoluble in water at 80° C., indicating that it was not volutin. Treatment of the opaque cells with dilute sodium bicarbonate obscurely revealed the presence of internal bodies in *Staphylococcus flavo-cyaneus* but not in *Staphy-*

lococcus aureus. With the high-voltage microscope cells of *Staphylococcus flavo-cyaneus* from a two-day-old culture at room temperature were transparent and contained beautiful, often paired, opaque bodies which, liberated from the cells by sonic vibrations, gave a position Feulgen reaction and closely resembled the nuclei previously observed in this organism by Knaysi (1941*c*, 1942). Unpublished results of chemical analysis made by Smolens showed that these bodies were rich in phosphorus and contained purine nitrogen. Cells from young cultures, observed with the high-voltage microscope,were either less transparent with poorly defined nuclei or homogeneously opaque. The cells of *Streptococcus pyogenes* became transparent but showed no internal differentiation, although it was reported by Sevag, Smolens and Lackman (1940) that they contained relatively large quantities of thymonucleic acid. On the basis of these observations Knaysi and Mudd concluded that bacteria contain nuclear material which, depending on conditions yet unknown but probably related to environment and development, may be diffused in the cytoplasm or may be partly or totally differentiated into a nucleus.

This hypothesis appeared to have confirmation in the observation by Knaysi (1946*b*) that forespores and young endospores stain homogeneously by the Feulgen procedure, but that mature spores show a positive granule or rod. However, in the light of later work by the author the interpretation given to this phenomenon, which appeared obvious at the time, may not be the only one. It is possible that this observation is more related to the chemistry of the endospore than to its nuclear structure; for instance it may mean that the ribonucleic acid of the forespore and young endospore is not as readily removed by hydrolysis as the ribonucleic acid of the mature spore; ribonucleic acid may be free in the mature spore and in a stable chemical combination in the forespore and young endospore.

Baylor, Appleman, Sears and Clark (1945) studied the internal structure of *Rhizobium leguminosarum* with and without previous treatment with various solvents and nuclear stains. Observation was made with the electron microscope. Untreated cells and cells stained with Heidenhain's hematoxylin contained poorly visible, opaque bodies. Hydrolysis with HCl, application of the Feulgen technique or staining by several other procedures caused distortion and increase in the opacity of the protoplasm. Only in cells treated with dilute sodium bicarbonate were the internal bodies clearly visible. These bodies were soluble in acetone and in 2% nitric acid. The authors concluded, however, that they may be nuclei. Nucleus-like bodies were observed in the colon-typhoid group by Peshkov (1945).

Bombardment of the cells of *Escherichia coli* with x-rays led Cavalli (1945) to conclude that the sensitive spot is smaller in old cells than in young ones, and that this explains the greater resistance of old cells.

Knaysi and Baker (1947) showed that the reason why the young cells of *Bacillus mycoides* usually stain uniformly and do not show internal differentiation when observed with the electron microscope is the presence of ribonucleic acid diffuse throughout the cytoplasm. Since the chemical composition and affinities of ribonucleic acid and of thymonucleic acid are similar, nuclei present in such an environment are masked, as would be a ball of glass immersed in a colorless liquid of equal refractive index. When the endospores of *Bacillus mycoides*, rich in ribonucleic acid, are placed in a medium containing a source of energy but no source of nitrogen, they germinate and grow, using their ribonucleic acid supply in the process. The vegetative cells which develop are transparent to the electrons, even at ordinary voltages, and each cell contains from one to six large opaque bodies which show evidence of division and play a leading rôle in the formation of the endospore. These bodies give the Sharp test for protein, a positive Fuelgen reaction and are distinct from any inclusions known to occur in the bacterial cell. They are demonstrable with methylene blue at a pH of 3.5 to 4.0, a property which they share with the nucleus of *Hansenula anomala* (Knaysi, 1946*d*). Although it was not possible to observe structural differentiation within these bodies, their microchemical reaction and particularly their behavior during the life cycle of *Bacillus mycoides* identify them as the nuclei of the cell. Knaysi and Baker presented evidence indicating that these nuclei do not divide by simple constriction.

For over half of a century cytologists have been observing in the cells of various bacteria bodies which, because of their tinctorial reactions or their occasional pairing or apparent constriction, were called nuclei. These criteria were critically examined by Knaysi

(1944) who pointed out that the only sure criterion for the nucleus is its behavior during the life cycle of the organism. The complexity of biological structures is such that no single chemical test or staining procedure can be relied on to determine the function of a structure. We are thus led to examine two procedures extensively used during the last decade, namely, the Feulgen reaction and the Piekarski-Robinow technique of staining with Giemsa's solution. In both procedures the cells are treated with acid, usually *n* HCl, for a given time at a given temperature. This treatment splits the purine bases from the thymonucleic acid, thus liberating aldehyde groups (Feulgen and Rossenbeck, 1924; Delaporte, 1939) and removes volutin or free ribonucleic acid (Knaysi, 1942, Figs. 16 and 17). A recent investigation by Vendrely and Lipardy (1946) shows that treatment with *n* HCl at 60° C. for ten minutes removes 37% of the desoxyribose nucleic acid from *Escherichia coli* and 7.5% of that substance from *Staphylococcus aureus*. It also removes most of the ribonucleic acid from both organisms. Since ribonucleic acid is responsible for the deep staining of the cytoplasm in yeasts (Knaysi, 1946*d*) and bacteria (Knaysi and Baker, 1947; Vendrely and Lipardy, 1946), its removal makes the cytoplasm poorly stainable, and unstainable at a moderately low pH. In the Feulgen reaction structures which are not removed by the acid treatment and which contain free aldehyde groups recolor Schiff's reagent. In the Piekarski-Robinow technique stainable structures which were not removed by the acid treatment become clearly demonstrable. In the bacterial cell it has been shown that the nucleus, the cytoplasmic membrane and its extensions, and certain presporal inclusions are not removed by hydrolysis and give a positive Feulgen reaction (Knaysi, 1944, 1945*a*, 1946*a*). Recent studies by the reviewer showed that the same structures also stain with Giemsa's solution, although more slowly than the nuclei. Confusion between nuclei and presporal granules has already resulted (Klieneberger-Nobel, 1945). There is also danger that bodies thus demonstrated may have been artifacts caused by coagulation and drying and wetting during the harsh manipulations to which the cells are subjected. For a first demonstration of a nucleus in bacteria the results of both methods could not be considered as a final proof, although both methods may be extremely useful when used discriminately on organisms of known nuclear structure. It must be added that the fundamental process in the Piekarski-Robinow technique is the treatment with acid. Giemsa's solution has excellent tinctorial qualities, but it could easily be replaced by basic dyes such as methylene blue (Lewis, 1942). In cells which have little or no ribonucleic acid, such as the transparent cells of Knaysi and Baker, the nuclei may be readily demonstrated by a modification of Burke's Gram-staining; the nuclei appear as Gram-positive bodies in a Gram-negative cell. Recently Lamanna (1947) observed and photographed nucleus-like bodies in germinating spores of *Bacillus cereus* stained by Burke's Gram-staining method, an unreported observation he had made earlier (1941) on *Bacillus subtilis.* During germination and for a few generations afterwards the ribonucleic acid content of the cell is considerably reduced (Dyar and Knaysi, 1947).

Since nucleus is a morphological concept, the nuclear nature of a body can be proved only by its behavior during the life cycle of an organism, a condition fulfilled only in the work of Knaysi (1942) with *Staphylococcus flavo-cyaneus* and in that of Knaysi and Baker (1947) with *Bacillus mycoides.* In the first case it was shown in addition that the bodies observed consisted of the nucleoprotein characteristic of the nucleus. In the second case the bodies observed had several of the microchemical and tinctorial properties of known nuclei. The fact that these bodies have not yet shown a chromosomal structure should not be given weight against their nuclear nature and their nuclear behavior: chromosomes may be present but unresolvable with the light microscope; chromosomes may be present but, because of the thickness and density of the bodies, they are not distinguishable with the electron microscope; finally, each body may consist of a single chromosome as assumed by Badian (1933, 1935) and by Robinow (1945).

Consequently a nucleus has been satisfactorily demonstrated in the cell of *Staphylococcus flavo-cyaneus* and in the cell of *Bacillus mycoides:* it will probably be demonstrated in other bacteria. On the other hand, it is not advisable to assume that all bacteria are similarly constituted until representative of all groups of bacteria are reinvestigated.

Cell Wall and Slime Layer

The Cell Wall. Although the existence of a cell wall in bacteria was demonstrated by Fischer (1891), this either was not generally known or was not generally accepted by bacteriologists. While studying the process of cell division, Knaysi (1941*a*) developed a simple method by which the cell wall can be clearly seen: the dried cell smear is fixed by heat, mordanted with a mixture of potassium alum and tannic acid, and examined under a film of carbol fuchsin. The cell wall appears blue, the cytoplasmic membrane dark red, and the protoplasm a lighter red. The cell wall is readily observed with the electron microscope and shows evidence of considerable ductility; when the cells are broken by sonic vibrations, the fragments of the cell wall appear jagged, indicating a certain degree of rigidity (Mudd, Polevitzky, Anderson and Chambers, 1941). Rigidity of the cell wall was also demonstrated in *Spirillum volutans* (King and Beams, 1942). The cells of this organism do not break up, nor are they greatly distorted by ultra-centrifuging. The intracellular inclusions are concentrated in the "centrifugal troughs" of the body, and the centripetal "crests" appear homogeneous. Elasticity of the cell wall was also shown by microdissection (Wámoscher, 1930). Robinow (1945) extended a method previously used by Gutstein (1924) for the demonstration of the ectoplasm: the cells are first stained with Giemsa's solution, mounted in a drop of water and a suitable field photographed; only the cytoplasm is said to be stained. The preparation is then removed, decolorized with acid alcohol, mordanted with 5% to 10% tannic acid, and restained with 0.02% of crystal violet in water; this is said to stain only the cell wall. The same field previously photographed after staining with Giemsa's solution is found, photographed, and the cells compared. The pictures published by Robinow leave more than a passing suspicion that the structures stained by crystal violet represent more than the cell wall. Recently Dyar (1947*a*) developed, in the reviewer's laboratory, a method in which the cells are treated with a cationic surface-active mordant, cetyl pyridinium chloride, the cell wall stained with Congo red, the cytoplasm with methylene blue. According to Dyar (1947*c*), this method was more successful with spirochetes than the two methods described above.

Dyar (1947*c*) determined cell wall resistance to hydrolysis with *n* HCl at 100° C. The wall of *Bacillus cereus* required 40 minutes for complete disintegration; those of *Bacillus mycoides* and *Bacillus subtilis* required 30 minutes; that of *Staphylococcus aureus* required 12 minutes and that of *Escherichia coli* 10 minutes.

The Slime Layer. The slime layer may be demonstrated with Knaysi's cell wall stain. It appears as a pale red layer of variable thickness surrounding the blue cell wall (Knaysi, 1943*a*). With the electron microscope the slime layer is not usually visible, but may be demonstrated when the cell suspension is prepared in a small droplet of water directly on the collodion film, or when a suitable mordant is used. Under these conditions the slime layer is better preserved and its density is increased (Mudd, Heinmetz and Anderson, 1943*a;* Knaysi, 1944). In homologous serum the slime layer binds some components of the serum and increases both in density and in thickness (Mudd, Heinmets, and Anderson, 1943*b*). The swelling reaction of the pneumococcus was quantitatively studied (Mudd *et al.,* 1943*b:* Johnson and Dennison, 1944). Under special nutritional conditions the thickness and density of the slime layer may be so increased that its demonstration with the electron microscope becomes immediate, and sometimes it may entirely mask the cell.

With respect to chemical composition the slime layer may be of two general types. The first is nitrogen free, as in *Rhizobium leguminosarium* and *Klebsiella pneumoniae;* the second is nitrogen-containing, as in *Bacillus anthracis* (Kramár, 1921). Capsular material is made up of large complex molecules; in the pneumococcus these molecules are filamentous. Within either type there is variation, not merely in spatial configuration, but also in the products of hydrolysis. Indeed either type may be found in different strains of what is considered the same species (Heidelberger, Kendall, and Scherp, 1936). The capsule of *Bacillus mesentericus* and of *Bacillus anthracis* (Ivanovics, 1938, 1939; Hanby and Rydon, 1946) and the extracellular slime of *Bacillus subtilis* (Bovarnick, 1942) are said to be polypeptides of d-glutamic acid.

It was suggested (Etinger-Tulczynska, 1933; Mudd, Heinmetz, and Anderson, 1943*a*) that a distinction should be made between true capsules and extracellular slime. The slime layer is not an integral part of the cell; its formation is considerably affected by the environment (Ivanovics, 1937; Morgan and Beckwith, 1939; Hoogerheide 1939, 1940; also a number of personal observations), and it may be removed without fatal injury to the cell (Dubos and Avery, 1931; Sickles and Shaw, 1933; Shaw, 1937; Dubos, 1939; McClean, 1941, 1942). Bacterial strains without a demonstrable slime layer may be induced to form large capsules by dissociation procedures (Knaysi, 1933).

Medical Aspects. In pathogenic organisms the cell wall and slime layer protect the bacterial cell against body fluid and are thus factors in virulence; the binding by the capsule of *Micrococcus pneumoniae,* and by the cell wall of *Eberthella typhosa,* of components of the homologous serum (Pijper, 1938, 1941; Mudd and Anderson, 1941; Mudd, Heinmets and Anderson, 1943*b*) is direct evidence for that action. It was also shown that removal of the capsule gives some protection to the animal. The cell wall and slime layer are important in diagnostic procedures, since the serological specificity of bacterial strains and the antigens responsible for agglutination are said to be located at the surface of the cell. In the words of Mudd (1944), "Type specific serologic reactivity, on which specific antibacterial immunity depends, may be determined by configuration either of a carbohydrate or of a protein component. The capsular polysaccharides of the various pneumococcus types are the classic examples of carbohydrate antigens which determine type. The surface somatic proteins ('M substance') of the various types of *Streptococcus* pyogenes are an example of type determination by protein antigens. In the case of the typhoid-paratyphoïd-dysentery group, type specificity is determined by a phosphorus-containing polysaccharide-protein complex in which the carbohydrate component is not type specific. The characteristic antigen reactivity (and toxicity) of the somatic antigens of the group appear, however, to be a property of the intact phosphorus-containing polysaccharide-protein complex and not of either the isolated carbohydrate or protein component alone. In all cases known to me, however, the reactive configurations which determine type specificity and antibacterial immunity are peripheral, *i.e.*, are in the capsule, as in pneumococci, in the bacterial cell wall, as in *Streptococcus pyogenes* and the Shigellas, or in the bacterial cell wall and flagella, as in *Eberthella typhosa* and the Salmonellas." A review of the literature on this phase of cyto-immunology may be found in the paper by Mudd from which this quotation was taken and in Dubos (1945).

Physico-Chemical Analysis of the Cell Surface. Electrophoretic mobility has long been considered a function of the cell surface, and

Abramson, Moyer and Gorin (1942) recommended use of the microscopic method for studying the surfaces of living cells. Dyar and Knaysi (1947) showed that the electrokinetic potential of the bacterial cell is an exclusive property of its surface and is not affected by change in the composition of the protoplasm.

Moyer (1936) found that electrophoretic mobility in young cells of *Escherichia coli* was lower than in mature cells; the mobility of the smooth strains remained constant throughout a long range of pH, whereas that of the rough strains varied with the pH. He also noted that the changes of mobility of rough or smooth strains with the concentration of electrolytes were similar to those of some carbohydrate and lipoid surfaces. Stearns and Roepke (1941) observed differences in mobility between rough and smooth strains of *Brucella abortus* similar to those observed by Moyer in *E. coli*. Bradbury and Jordan (1942) concluded from electrophoretic measurements that sulfanilamide and related drugs behaved, at the cell surface of *E. coli*, as did *p*-aminobenzoic acid, and that the drugs associated with the cell through their aromatic amino groups. Cohen (1945) detected imidazole and amino groups at the cell surface of *Proteus*. Dyar and Ordal (1946) compared the electrokinetic behavior of representatives of various groups of bacteria, a myxococcus and a spirochete, with the behavior of known particles. They concluded that the chemical nature of a substance present at the cell surface may be detected by its electrokinetic behavior in the presence of surface-active agents. In the reviewer's laboratory Dyar (1947*b*, 1947*c*) found that the "lipids present in the surfaces of living bacterial cells can be measured somewhat quantitatively. The amount of lipid in the surface of cells of *Micrococcus aureus* and *Mycobacterium smegmatis* can be altered at will, within limits, by varying the conditions of growth. Some other bacteria such as bacilli do not produce surface lipids even though the cytoplasm is full of fat. . . . Amphoteric material in the surface of *Micrococcus aureus,* distinct from the lipid, was detected by change of mobility with pH. . . . Some of the properties of the cell wall 'skeleton' of *Micrococcus aureus* remaining after lipase and trypsin treatment were determined. They indicate, but do not prove, that it may be of carbohydrate nature although unlike either cellulose or the insoluble dextran synthesized by some streptococci".

MOTILITY

Motile bacteria may be divided into two groups: those which creep slowly on solid surfaces or the surface film of liquids, and those which swim freely throughout a liquid. Creeping motion is observed only among the large sulfur bacteria and myxobacteria. It is accomplished by periodic deformation of the cell (Ullrich, 1926). Certain peculiarities in the motion of myxobacteria on the surface of agar were observed by Stanier (1942). In the swimming group, cells of the bacteria have filamentous appendages, called "flagella", generally considered the organs of locomotion. The spirochetes are generally considered to move by deformation of the cell. These general conceptions, apparently long established in bacteriology, were recently shaken by the discovery of flagella-like appendages in the spirochete *Treponema* (Wile, Picard and Kearney, 1942; Morton and Anderson, 1942; Wile and Kearney, 1943; Mudd, Polevitzky and Anderson, 1943) and by recent observation of Pijper (1946, 1947*a*) of motile cells of *Eberthella typhosa* apparently without flagella. On the other hand, Lofgren and Soule (1945) reported that the flagella-like extensions they observed in *Spirochaeta novyi* were the result of the mechanical action of distilled water and centrifugation; in the case of bacteria the challenge of Pijper's conclusion that flagella are threads of slime which are the result, not the cause, of motion has not yet been met; on the contrary, the recent observations of Hillier and Knaysi (1947*a*) on the origin of flagella in *Bacillus mycoides* appear to be in partial agreement with the corresponding phase of Pijper's hypothesis. It is not generally known, however, that students of the flagellates do not agree on the specific rôle of the flagella in the motion of these organisms (Metzner, 1920; Lowndes, 1941; Brown, 1945). Regardless of the outcome, Pijper's hypothesis has stimulated thought and research which will doubtless lead to a better understanding of the nature and function of the flagella and of applications in taxonomy and immunology.

Flagella are not necessary for the life of the cell (Lominsky and Lendrum, 1942). They may be lost by dissociation (Neri, 1940), and their formation is somewhat affected by the environment (personal observation). They adsorb antibodies from a homologous serum with a consequent increase in thickness and stiffness, and the formation of loosely held groups upon agglutination (Pijper, 1938, 1941; Mudd and Anderson, 1941). They agglutinate with acid at about pH 4.4 (Malek, 1938; Ogonuki and Miyata, 1940). Their chemical nature was studied by Boivin and Mesrobeanu (1938).

Other papers of interest are that of Ôgiuti (1936) in which the effect of pH, temperature, osmotic pressure, viscosity and homologous serum on the velocity of various bacteria was studied; that of Hutchinson and McCracken (1943) on a sulfur bacterium; that of Johnson, Zworykin and Warren (1943) on luminous bacteria; and that of Hofer (1944) on *Azotobacter.*

INCLUSIONS

Studies with the ultracentrifuge led King and Beams (1942) to conclude that all demonstrable inclusions of *Spirillum volutans* have higher specific gravity than its cytoplasm.

Lipoid Inclusions. It was shown by Knaysi (1945*a*) that the lipoid inclusions formed by *Bacillus cereus* in the presporal stage give the Sharp test for protein a positive Feulgen reaction. More recent observations also showed that they stain like the nuclei, although somewhat more slowly, with Giemsa's solution. This should explain the high specific gravity of the fatty inclusions of *Spirillum volutans* observed by King and Beams. Knaysi observed the formation of these inclusions in the cytoplasmic membrane and their elimination into the cytoplasm. They are not used up when the cell is transferred to a fresh medium, but are distributed in the progenies of that cell. They are formed even in very poor media, and their size is not greatly influenced by the composition of the medium. Burdon (1946) found that "stainable fatty material in the form of cytoplasmic inclusions, or such material associated with structural elements of the cells, is present in all fungi and in the great majority of bacteria, whether these are aerobic or anaerobic, saprophytic or parasitic, pathogenic or nonpathogenic. . . . With certain notable exceptions (*e.g., Alcaligenes fecalis* and *Azotobacter*), fatty material was found to be more abundant and to occur with greater regularity in Gram-positive bacteria than in Gram-negative bacteria. An apparent tendency for saprophytic species to contain more fat than parasitic species was noted. . . . Although media of special composition are required for the appearance of stainable lipid in the case of certain bacteria, the formation of fatty inclusions was shown not to be dependent upon the presence of glucose, glycerol, or other fermentable carbohydrate in the medium". Burdon observed a remarkable constancy in the location of these inclusions within the cell in a given genus and sometimes in a given species. Knaysi (1946*d*) found that the lipoid inclusions of the yeast *Hansenula anomala* "are noticeably utilizable only by volutin-free cells, in an environment devoid of nutrients, and under strongly aerobic conditions, both as a source of energy and a source of nitrogen". There was no evidence that they were used up during active growth in complete nutritive media; "on the contrary, the rate of their formation in such media is highest during active growth". Starkey (1946) found a definite relation between lipid content of certain soil yeasts and the composition of the medium.

Volutin. Delaporte (1939) showed that volutin is essentially ribonucleic acid. It can be removed from the cell with 0.02% sodium bicarbonate solution, but some protein was always present in the extract. Knaysi (1946*d*) found that the volutin inclusions of *Hansenula anomala* were readily utilized as a source of nitrogen but not of energy. In a glucose solution the volutin is used up and the stainability of the cell is reduced so that the nucleus may be demonstrated by simple methylene blue staining. In complete media volutin accumulates during active growth and is used up in old starving cultures. Knaysi and Baker (1947) found that ribonucleic acid is present in high proportion in the endospore of *Bacillus mycoides.* In a medium containing a source of energy but not of nitrogen, the spores germinate, using up their supply of ribonucleic acid. The vegetative cells developed have a poorly stainable cytoplasm and are transparent to electrons at ordinary voltages, except for the nuclei which become readily demonstrable by simple staining or with the electron microscope. Knaysi (1947*a*) showed that cells thus developed are highly permeable. Apparently the loss of ribonucleic acid from the cytoplasmic membrane loosens the structure of that membrane. Cells grown in ordinary media contain ribonucleic acid diffuse in the cytoplasm, and probably present in the cytoplasmic membrane, which is responsible for the uniform staining of these cells and their uniform opacity to electrons. The cells of *Bacillus mycoides* do not usually deposit volutin, and the ribonucleic acid is probably present in combination with other protoplasmic constituents, since it is not removable with mild alkalis. It is removable by hydrolysis with *n* HCl, as in the Piekarski-Robinow technique (Vendrely and Lipardy, 1946). Knaysi (1942) had shown that volutin does not interfere with the Feulgen reaction because it is removed by hydrolysis with *n* HCl.

VEGETATIVE REPRODUCTION

In young cultures and in ordinary media, normal common bacteria reproduce almost always by a process known as "cell division". In old cultures, and in young cultures under special conditions, budding may be rarely observed. Frequent budding was observed by the reviewer in *Azotobacter indicum* and in a pneumococcus strain. Cells which have undergone considerable change of form and size may reproduce by fragmentation, a process which is a mixture of aberrant cell division and budding.

Cell Division. Details of the process of cell division were observed by Knaysi (1941*a*). A ring grows from the cytoplasmic membrane inwards, separating the cytoplasm into two parts. This cell plate is similar to the cytoplasmic membrane in chemical and physical properties. The cell plate is then split, apparently inwards. Sometimes the separation is not complete, and the sister cell plates may still be attached in the center; in dried cells, shrinkage stretches this attachment into a plasmodesma. Separate cell walls are formed, leisurely, for both sister cells. In typical cases division is equational. However, asymmetric cell division is occasionally observed in young culture and more frequently in old ones. Knaysi (1933) developed a strain of *Bacillus megatherium* in which asymmetric cell division was strikingly common in young cultures.

Description of the process by Robinow (1945) is very similar to that given by Knaysi (1930, 1941*a,* 1944). The only difference is that Knaysi stated that a cell wall is formed independently by each sister cell, and Robinow (1945, p. 376) was not sure whether the cell wall is "double from the beginning or first laid down in single thickness". Therefore, the statement: "Knaysi . . . concluded . . . that bacteria divide by a retraction of the cytoplasm in opposite direction from the middle plane" (p. 371) must be overlooked. Division by "retraction of the cytoplasm" had been described earlier by Knaysi (1929) for *Mycobacterium tuberculosis,* but his later studies (1941*a*) showed that this organism divides like other bacteria. In *Schizosaccharomyces pombe* also Knaysi (1941*a*) had found that the cytoplasm is directly split by the formation of cell walls without previous formation of a cell plate by inward growth of the cytoplasmic membrane, as in the bacteria, and Robinow really confirmed this observation by stating: "Cytoplasmic cell boundaries or cell plates which, in bacteria, are usually laid down before the formation of transverse cell walls were not observed in the fission yeast". It is indeed incomprehensible why, a few lines before, Robinow wrote: "For a different opinion see Knaysi (1941)". This difference between the division of bacteria and yeasts was one of the chief points reported for the first time, made clear and repeatedly emphasized in Knaysi's paper. Recent unpublished observations on the smooth strain C_2 of *Bacillus mycoides,* made by Knaysi and Hillier with the electron microscope, show that the centripetal split of the cell plate is usually brought about by centripetal growth from the lateral wall, and that the new cross wall is differentiated into two layers often before its growth is complete. Severance of the sister cells is apparently brought about by the pressure resulting from their growth and superficially simulates simple constriction.

Budding. No detail study of budding in bacteria was made. The reviewer's preliminary observations on *Azotobacter indicum* indicate that it proceeds from a broad base, often suggesting a process intermediate between budding and cell division.

Fragmentation. By fragmentation is meant the apparently irregular break-up of a cell into smaller ones with or without simultaneous or subsequent growth. The break-up may be accomplished by a cytological process indistinguishable from cell division, as in *Bacillus megatherium,* or by a combination of division and budding, as in the large bodies of Dienes.

The "Large Bodies" of Dienes. In a series of papers Dienes (1939, 1940, 1942, 1943, 1944, 1945, 1946, 1947) described what he considers a complex reproductive process in bacteria, particularly in Gram-negative ones. It consists of "the swelling of bacteria into large, round bodies and the reproduction of bacteria either directly in them or by the intermediary of a soft granular growth (L_1) . . .". This process was observed chiefly in freshly isolated pathogenic bacteria in cultures or when cells from the proper strain were transferred to water. In *Proteus* the process was observed regularly "when spreading cultures of appropriate strains come in contact". No union of cells was involved. Dienes suggests that this may indicate a sexual process. Dienes and Smith (1944) observed that in a strain of *Bacteroides* "The bacterial filaments segment and the large bodies develop from the neighboring ends of two segments. This process suggests an autogenous conjugation, examples of which are well known in fungi".

It is not possible at the present time to give this process a final interpretation. The formation of normal bacterial forms or L_1 forms from the large bodies does not seem to take place by what is known as "free cell formation", but by budding and fragmentation. This much is clear from the series of observations published by Dienes and particularly from the slides shown by Smith, Hillier and Mudd (1946). The reviewer is convinced that the swelling of bacterial cells is always associated with a change in permeability and with osmotic relationships. When *Bacillus mycoides* is grown in glucose broth containing 0.5 molar $MgSO_4$, numerous swollen cells, similar to the "large bodies" of Dienes, are observed, particularly during an early period of cultural development. At later stages these swollen cells, known as "plasmoptysis figures", are reduced in number, and normal cells predominate. According to Stapp and Zycha (1931), the swollen cells are unable to reproduce. The reviewer's observations with *Escherichia coli* are of special interest. When the cells are transferred from an ordinary medium to one containing 0.25 to 0.50 molar $MgSO_4$, they are at first plasmoylzed; many of the cells are subsequently deplasmolyzed and grow, forming a highly pleomorphic culture consisting of thick curved cells with abruptly tapering ends, of ring forms and of swollen cells of various forms, resembling a culture of a pleomorphic yeast. All types of cells are vacuolated. The swollen cells are often irregularly partitioned, and budding is frequently observed; the explosive separation of a segment and its reunion with the mother cell were sometimes observed, indicating softness or possible absence of a cell wall. In certain cases one gains the impression of the break-up of one of the giant cells into smaller irregular ones, but this was not actually observed. Dienes also observed the formation of "large bodies" in response to toxic influences, but these did not reproduce. The reviewer's observations indicate that enlargement of the cells is not due to mere swelling, but chiefly to actual growth.

In view of the close similarity between these observations and those of Dienes', the phenomenon may be considered a response to the environment. The influence of one *Proteus* strain on another may be readily attributed to mutual antagonistic influences. Sexual stimulation without actual fusion is known in the lower fungi, but such an interpretation should be postponed until more is known about the cytological processes involved. The distinction between large bodies "normally" formed and large bodies formed in response to toxic influences on the basis of ability to multiply may not be desirable, since the giant cells of *Escherichia coli* formed in the presence of $MgSO_4$ show a tendency to multiply by forming irregular plates. The difference may be in the magnitude and nature of the "toxic" influence. The morphologic peculiarities of the L cells is due to the manner in which they are formed and to softness of the cell wall; it seems that when the cell is deformed as the giant cells are, polarity of the cell is affected or temporarily lost, and it reproduces vegetatively by forming plates of division in indefinite directions and breaking up into smaller pleomorphic units. These units should not be called "gonidia", since the term, as now understood, involves free cell formation.

THE ENDOSPORE

Cytology of Formation. a) Growth from a single granule. This was described by Hoyt (1935) in *Clostridium septicum,* but examination of his photographic record indicates that his granules may well have been forespores formed by "condensation of the protoplasm".

b) Condensation of the protoplasm. This includes those methods in which the endospore is said to be initiated by the sudden appearance, in a previously differentiated part of the protoplasm, of a primordium or a forespore with dimensions exceeding, equalling or slightly below those of the mature spore. It was described by Delaporte (1934, 1935, 1936) in *Oscillospira guilliermondi* and *Oscillospira batrachorum* of the Cyanophyceae, and in *Spirillum praeclarum;* by Roberts (1942) in *Clostridium pasteurianum;* and by Knaysi (1946*c*) in *Bacillus cereus.*

Nuclear Behavior. Allen, Appleby and Wolf (1939) observed three methods of spore formation in a bacillus isolated from grass. "The main changes involved are explained on the assumption that the normal vegetative cell is haploid and contains one chromosome. Prior to one type of spore formation a diploid cell is thought to be formed by division of the chromosome, accompanied by a failure in cell division. Meiosis there occurs, resulting in the formation of a well-marked, deeply staining tetrad, followed by separation of the chromosomes and possibly a second mitosis. One of the resultant chromosomes is involved in the formation of the spore, and the remainder appear to be extruded. Klieneberger-Nobel (1945), using the Piekarski-Robinow technique, confirmed the observations of Badian (1933, 1935) which, like those of Allen *et al.*, attribute to the vegetative cell a haploid state followed by autogamy and two nuclear divisions (reduction division). Only one of the resulting chromosomes forms part of the spore; the other three disintegrate.

Knaysi and Baker (1947) observed with the electron microscope spore formation in cells of *Bacillus mycoides* made transparent by growth in a nitrogen-free medium. Their observations suggest that the nuclei of the potential sporangium gather into two groups of one to three nuclei each. The two groups are formed at a certain distance from each other, and an area, slightly denser than the cytoplasm of the mother cell, appears to grow from each nucleus, or groups of nuclei, toward the other; the forespore results from the merging of those two areas, and its length is determined by the relative position of the two nuclear groups. In non-filamentous growth no nuclei were left out to disintegrate in the sporangium, and there was no evidence of nuclear fusion before or after the formation of the forespore, although the authors do not exclude the possibility. At first the nuclei occupy polar positions, but later may be seen anywhere in the spore.

In the light of this research it seems that Badian and his followers were confused by the presence of presporal inclusions. In *Bacillus cereus* these inclusions originate in the cytoplasmic membrane and are eliminated to the cytoplasm. They give a positive Feulgen reaction and take nuclear stains, although more slowly than the nuclei (Knaysi, 1945*a*, 1947*b*). Imšenecki (1945) observed lipoprotein granules in all aerobic sporeformers and stated that under certain conditions these granules stain with basic dyes and have been mistakenly considered nuclei.

Structure of the Endospore. Delaporte (1939–40) observed a refractile membrane, a hyalin cytoplasm and a Feulgen-positive rod or granule. Robinow (1945) concluded from observation of hydrolyzed spores of various bacilli that the endospore consists of "three concentric layers. . . . The central portion, a narrow core, is refractile and stainable, and merges into a thick layer of less refractile and less readily stainable cytoplasm, bounded on its outer surface by a thin crust of stainable, non-refracting material which may be identical with the spore membrane. . . . No chromatinic matter is found in the cytoplasm". He described a biconcave nucleus, "the most refracting element in hydrolyzed spores; it is attached to the outer surface of the cytoplasm." Stille (1937) had observed a single Feulgen-positive body in the endospore and stated that the youngest vegetative cell contains two or four similar bodies.

The work of Knaysi (1945*d,* 1947*b*) with the light microscope and that of Knaysi and Baker (1947), Knaysi, Baker and Hillier (1947) and Hillier and Knaysi (1947*b*) with the electron microscope show that the endospore of *Bacillus mycoides* consists of a double membrane, an inner rigid one and an outer elastic one, enclosing a cytoplasm rich in ribonucleic acid and in lipoidal material diffuse throughout; the nuclei, usually two or more, most commonly occupy polar positions. The reviewer has been able to satisfy himself that the shape of the nucleus and its extra-cytoplasmic position observed by Robinow are artifacts caused by a combination of incipient germination and hydrolysis. Recent work (Knaysi and Hillier, 1947) with *Bacillus megatherium* showed that this organism sheds a single coat consisting of long, beaded, parallel molecules cemented together by a substance which disappears soon after germination. It is not known whether or not an inner coat existed in the resting spore and was used up during germination.

Cytology of Germination. Lamanna (1940) made a careful study of spore germination in *Bacillus* and found correlation between the mode of germination, spore antigen and several physiological properties. Knaysi and Gunsalus (1944) confirmed Lamanna's work with the Marburg strain and the Ford and Lawrence strain of *Bacillus subtilis;* the latter strain was renamed *Bacillus licheniformis* by Gibson and Abdel-Malek (1945). Details of spore germination in *Bacillus mycoides* were studied with the electron microscope by Knaysi, Baker and Hillier (1947); the spore is surrounded by two coats which become bent and split equatorially. Lateral splitting of the spore coat was recently observed by Knaysi and Hillier (1947) in a strain of *Bacillus megatherium* heretofore considered to germinate by absorbing its coat. Only one coat is shed. The possible existence of a second coat and its utilization during germination are considered, but no evidence for the existence of such a coat has yet been found.

Antigenic Structure. Krauskopf and McCoy (1937) found a close serological relationship between spores and vegetative cells. On the other hand, Lamanna (1940*b,* 1940*c,* 1942*b*), Howie and Cruickshank (1940) and Bekker (1944–45) were able to demonstrate antigens characteristic of the spores, and Lamanna was able to classify members of the genus *Bacillus* on the basis of their characteristic spore antigens. The observation of Dyar in the reviewer's laboratory that spores have a different electrophoretic behavior from vegetative cells is a confirmation of the existence of surface antigens characteristic of the spores.

Chemical Composition. Little is known about the chemical composition of the endospore other than that it contains a number of enzymes (Effront, 1914; Ruehle, 1923; Cook, 1931; Tarr, 1933; Virtanen and Pulkki, 1933) and that it contains more bound water and less free water than the vegetative cell (Friedman and Henry, 1930; Henry and Friedman, 1937). Curran, Brunstetter and Myers (1943) demonstrated Ca, K, Cu and Mn by spectrometric analysis. Knaysi (1945*d*) demonstrated ribonucleic acid diffuse throughout the cytoplasm and utilizable as a source of nitrogen and phosphorus but not for energy. It may be added that the cytoplasm of the spore is rich in lipoid material, also diffuse. Since the spore contains nuclei, it also contains the nucleoproteins characteristic of the nucleus.

Effects of the Environment on Spore Formation. Although the endospore is a part of the life cycle of certain bacteria, its formation is considerably affected by the environment. Loss of the ability to form endospores, with or without regaining that property, was reported by Knaysi (1933, 1935*a*); the effect of dissociation on the relative number of spores in a given culture was studied by Hayward, Marchetta and Hutton (1946). The environmental factors investigated during the last decade were the following: the temperature of incubation, composition of the medium with respect to type and initial concentration of gross nutrients, molecular oxygen, salts, pH, nutrilites; the accumulation of by-products; gradual drying of the medium; position of the cell in the colony (Greene, 1938; Kaplan and Williams, 1941; Roberts and Baldwin, 1942; Hayward, 1943; Knaysi, 1945*c;* Knaysi and Baker, 1947). It was shown by Knaysi that these factors are not independent variables but are interrelated by their specific effect on the utilization of food and the health of the cell. Knaysi concluded that, with *Bacillus mycoides,* spores are formed by healthy cells facing starvation, and the ideal environment for sporulation is that in which food is completely utilized without accumulation of by-products. Whether or not there are organisms stimulated to sporulate by the accumulation of metabolic products remains to be shown. Sporulation has an optimum temperature and a sharp optimum pH; it does not usually require nutrilites other than those required for growth, but nutrilites may exert an indirect stimulating effect; for instance, vitamin B_1 may promote sporulation, in a medium where acid products of metabolism tend to accumulate, by its decarboxylic activity which prevents a significant drop in pH. However, it was recently reported by Dutky (1947) that he developed several media for the growth of *Bacillus popilliae* and *Bacillus lentimorbus;* these media "gave consistently high yields of vegetative forms, but none was adequate for sporulation". Whether this indicates need for an additional growth factor is not clear. Drying of the medium is detrimental to sporulation by *Bacillus mycoides* except when drying significantly increases oxygen supply.

An interesting effect of the temperature of incubation was reported by Christian (1931) on a form of *Bacillus* isolated from heated milk. The optimum temperature for the growth of this organism was between 30° and 37° C. and the maximum for its sporulation 22° C. At 37° C. the organism shows "evidence of an effort at spore formation. The cell contents concentrate in a terminal position, as in the case of the formation of true spores, but no spore wall is formed. Later, these terminal bodies or 'abortive spores' are liberated and persist in the cultures in the free state for long periods. It has not been demonstrated whether they are capable of developing further under suitable conditions or of giving rise to new generations of the organism". One wonders whether or not similar bodies recently observed in certain thermophilic bacteria (Buck, 1947, and personal communication) are abortive spores which may attain full development below the optimum temperature for vegetative growth.

Effect of the Environment on Spore Germination. Structurally and chemically, the normal endospore is a complete cell, and when it is placed in a suitable environment, it proceeds to germinate. However, the time it takes a given spore to germinate varies considerably with the environment; in a given environment spores of a given culture do not all germinate at the same time. Delayed germination or dormancy observed chiefly among heated spores, and to a certain extent in unheated spores, is probably due to injury or deficiency, and the number of "dormant" spores decreases as the quality of the medium in which they are planted is improved (Morrison and Rettger, 1930; Curan and Evans, 1937; also personal observations). Indeed a normal spore may germinate in an environment containing the necessary nutrient even when this environment is otherwise detrimental to the survival of the germ cell (Knaysi, 1945*d*).

With respect to concentration of food Curran (1931) found that in peptone solutions the minimum concentration for germination is 0.025%; in 10% peptone there is considerable retardation of germination. Knaysi (1945*d*) observed no germination of *Bacillus mycoides* in distilled water or in solutions of various salts, but there were germination, growth and spore formation in bacto agar prepared with distilled water without the addition of any nutrients. Germination was also observed in a solution of 0.2% glucose and 0.1% potassium phosphate, but not when the glucose was replaced by lactose, a sugar not fermented by the organism. It was concluded that the endospore contained a relatively large quantity of a substance which is an excellent source of nitrogen but no source of energy. Knaysi and Baker (1947) identified the substance as ribonucleic acid and showed that germination can take place in a solution of glucose buffered with Na acetate, indicating that ribonucleic acid is also a good source of phosphorus. It results from this work that there is no minimum concentration of the nitrogen or phosphorus sources which should be added to the medium, and that the minimum for the source of energy is below 0.2% of glucose. Evans and Curran (1943) and Curran and Evans (1945) showed that germination is favored by exposure of the spores to "sublethal heat". Knaysi (1947*a*) observed that germination in a glucose solution is favored by previous autoclaving of the sugar solution or by the addition of traces of phosphate. This effect seems to consist of activation of the sugar and is expressed by rendering reversible and by lowering of the oxidation-reduction potential (Wurmser, 1930; Knaysi, 1935*b*); autoclaving of culture media, or the addition of reducing substances, is known to be beneficial for the initiation of growth (Dubos, 1929; Allyn and Baldwin, 1930, 1932; Wood, Wood and Baldwin, 1935; Nelson, 1944). The beneficial effect observed when the spores are heated in distilled water may be attributed to activation of material inside of the spore.

Consequently the behavior of a normal spore in a given environment is better understood when one realizes that it is a cell, resting because of the lack of food or of the proper conditions for its utilization, containing a source of nitrogen, minerals and enzymes, but separated from its environment by a coat of low permeability.

Resistance of the Endospore. From the practical point of view the chief characteristic of the endospore is its ability to attain a high degree of resistance to drying, heat, chemicals and other deleterious agents. It should be emphasized, however, that the resistance of endospores varies considerably with the species; nevertheless, it is not a constant for the species. It varies considerably with the conditions under which the spores were formed (Theophilus, 1937) and in the same culture varies considerably from spore to spore (Hodge and Knaysi, 1937); a morphological basis for variation in resistance between spores of the same culture was supplied by Knaysi, Baker and Hillier (1947). The cause of spore resistance has been a subject for speculation. In the early decades of bacteriology it was attributed to the existence of a thick coat of low permeability. Later investigators attributed resistance to peculiar spore enzymes and other proteins or to a low water content. The rôle of the spore coat in resistance to chemicals can not be denied; it can be deduced *a priori* and may be concluded from the observation of Knaysi (1945*d*) that when glucose is fermented by spores, the pH reached is considerably below that reached when vegetative cells are used; that the fermenting spore gradually assumes vegetative characteristics without normal germination and is killed by the hydrogen ions. Some correlation was also found between toughness of the spore coat and resistance to moist heat

(Knaysi, 1938; Lamanna, 1937). The high resistance of the spores of thermophilic bacteria to heat (Esty and Williams, 1920) is undoubtedly due chiefly to peculiar enzyme systems and other proteins stable at high temperature; a correlation was also found by Lamanna (1942*a*) between the maximum temperature of growth and the resistance of spores to heat. There is no evidence, however, that in the same strain the spore enzymes, for instance, are different from those of the vegetative cell. Since the spore contains less free water than the vegetative cell (Henry and Friedman, 1937), this difference is probably an important factor in spore resistance to heat as well as to chemicals. In the reviewer's opinion the high concentration of ribonucleic acid and of lipoid material in the cytoplasm of the spore, the low free-water content of the spore and the low permeability of the coat, taken together, should be sufficient to explain spore resistance to drying, heat, chemicals, ultraviolet radiations and possibly other physical and chemical agents. (See also von Angerer, 1938.)

SEXUAL REPRODUCTION

Existence of a sexual mechanism in bacteria has been claimed by a number of bacteriologists (Schaudinn, 1902, 1903; Mellon, 1925; Löhnis, 1921; Enderlein, 1925; Hadley, 1928; Badian, 1933, 1935; Allen *et al.*, 1939; Klieneberger-Nobel, 1945; and many others). The evidence has never been satisfactory. Sherman and Wing (1937) developed substrains of *Escherichia coli* which differed from the parent strain in the ability to ferment certain substances. A strain which was sucrose +, salicin –, and one which was sucrose –, salicin + were inoculated into the same test tube and incubated together for one week. Strains which resembled the parent strains and others which were sucrose + and salicin +, sucrose – and salicin – were isolated from the mixture. However, since similar strains were also found without mixing of the strains, the authors refrained from giving their results sexual interpretation. Gowen and Lincoln (1942), using *Phytomonas stewartii,* drew a similar conclusion.

Recently, Lederberg and Tatum (1946) and Tatum and Lederberg (1947) performed fundamentally similar experiments with *Escherichia coli* and concluded that "Evidence has been presented for the occurrence of character recombination. . . . This suggests the existence of a sexual phase. Recombination of genes controlling several growth factor requirements and resistance to a specific bacteriophage have been found". The authors admit an alternative interpretation, assuming "the occurrence in the medium of transforming factors capable of inducing the mutation of genes, bilaterally". However, "Attempts at the induction of transformations in single cultures by the use of sterile filtrates have been unsuccessful. . . . The fusion presumably occurs only rarely, since in the cultures investigated only one cell in a million can be classified as a recombination type. The hypothetical zygote has not been detected cytologically". The evidence does not appear to us to be of a higher order than that presented by Dienes and Smith (1944) and by Dienes (1946).

STAINING

The Gram Stain. The structural, chemical and physical bases of the Gram reaction were discussed by Knaysi (1944). Dubos and Macleod (1938) reported that treatment of the heat-killed, Gram-positive cells of pneumococci with a polynucleotidase obtained from animal tissues rendered those cells Gram-negative. Henry and Stacey (1943, 1946) secured the same results with yeasts and several Gram-positive bacteria by the action of certain carbohydrate-splitting enzymes or by extraction with a solution of bile. Extraction of Gram-positive cells with a 2% aqueous solution of bile removes some carbohydrate and a substance identified as Mg-ribonucleate, leaving a Gram-negative skeleton rich in basic protein material. If this skeleton is treated with formaldehyde or certain other reducing agents and soaked in the extract, the Mg-ribonucleate recombines with the skeleton and the cells become Gram-positive again. Henry and Stacey concluded "that the essential constituent in Gram-positive organisms . . . lies in their having as part of their surface structure the magnesium salt of ribonucleic acid". Knaysi (1943*a*) showed that the volutin present in the vacuole of *Thiobacillus thiooxidans* was Gram-positive; volutin is known to consist of ribonucleic acid. Dubos (1945) was able to reverse the Gram reaction of pneumococci by treatment of the heat-killed cells with a solution of crystalline ribonuclease. Bartholomew and Umbreit (1944) confirmed the findings of Henry and Stacey and of Dubos. Bartholomew (1946) found that *Neisseria catarrhalis* was different from other Gram-negative bacteria by becoming Gram-positive when treated with extracts containing Mg-ribonucleate. Knaysi (1947*a*) observed that the vegetative cells of *Bacillus mycoides* developed from the spores in nitrogen-free media were Gram-negative except the nuclei. Dyar and Knaysi (1947) found that when a slant of meat infusion agar is inoculated with a spore suspension of *Bacillus mycoides,* the germ cells and subsequent generations of vegetative cells during the first few hours are, in the majority, Gram-negative.

In view of the observations reported above, it may be asked whether or not it should be concluded that the mechanism of the Gram reaction is exclusively chemical and bound with Mg-ribonucleate, or whether permeability also plays a rôle. Since removal of ribonucleic acid changes the permeability of the cell, even of living cells developed in a nitrogen-free medium, it can not be concluded that permeability does not play a rôle in the Gram reaction. On the other hand, in cells partially autolyzed the cytoplasmic membrane remains strongly Gram-positive. Also when the cells are broken up mechanically, or when the cytoplasmic membrane is partially removed by chemical action, fragments of this membrane remain strongly Gram-positive, although the cytoplasm becomes Gram-negative. It seems, therefore, that the Gram reaction, as applied to the entire cell, is a property of the cell. It is interesting that Henry and Stacey stated that Mg-ribonucleate confers Gram-positiveness only when it combines with complex molecules of the cell surface, meaning the cytoplasmic membrane. It seems that ribonucleic acid exerts both a direct chemical action and an indirect action by affecting permeability of the cytoplasmic membrane. It may also be added that, although ribonucleic acid is the chief substrate of the Gram reaction, the fact that the nuclei of cells developed in a nitrogen-free medium are also definitely Gram-positive indicates that other cell material may contribute to the reaction in the complete cell.

The Acid-fast Reaction. Reeves and Anderson (1937) and Stodola, Lesuk and Anderson (1938) isolated from the "unsaponifiable wax" of the tubercle bacillus a hydroxy methoxy acid which was strongly acid-fast. This acid was named "mycolic acid". Yegian and Porter (1944) showed that "non-acid-fast forms appearing in stained smears of young cultures of the tubercle organism are created by mechanical action of the platinum loop or the spatula used to disperse the organisms" and that sectioning may also destroy acid-fastness. Yegian and Baisden (1942) and Porter and Yegian (1945) studied the factors which affect beading in the tubercle bacillus and concluded that the Much granules and beads are artifacts. Lamanna (1946) studied the mechanism of beading in mycobacteria stained with Ziehl-Neelsen's carbol-fuchsin and concluded that it is "the result of phenol and dye separating out as a liquid phase" and that "the basis for the acid-fast property lies in the greater solubility of the phenol and dye in the cell constituents than in the decolorizing agent".

CYTOLOGY AND CHEMOTHERAPY

The importance to medicine of a sound knowledge of the structural make up of the bacterial cell and of the properties and behavior of these structures has been realized by a number of eminent bacteriologists (Mudd, 1944; Dubos, 1945; Pijper, 1947*b*), and a number of medical relationships were briefly indicated in previous sections of this review. Of these relationships none has greater potentialities in practical application than those of structure to virulence and resistance of the bacterial cell. In the field of chemotherapy emphasis is placed on metabolic processes, and little consideration is given to the intimate relation between metabolic activity and structure; if loss of the capsule reduces the virulence of pneumococci, then it may be possible to alter the environment so that capsular material is not synthesized. Since some of the permanent structures of the cell do not have a fixed chemical composition, it should be possible by altering the environment to modify the chemical and physico-chemical properties of these structures so that the virulence and resistance of the cell are decreased. When the endospores of *Bacillus mycoides* germinate in a nitrogen-free medium, the vegetative cells which develop are Gram-negative and very permeable to neutral red, even in very dilute solutions. In these cells the cytoplasm and the cytoplasmic membrane are altered in chemical composition; they are practically free of ribonucleic acid and possibly of other substances. Loss of ribonucleic acid from the cytoplasmic membrane seems to increase its permeability; loss of that substance from the cytoplasm should deprive the nucleus and other important components of the protoplasm from a considerable measure of protection against drying, chemicals, ultraviolet light and other harmful agents; the cells should be more readily killed by components of animal fluids and by chemotherapeutic agents. In this case synthesis was prevented by deficiency of the medium. If synthesis can be partially or totally inhibited by the addition of some substance to a nondeficient medium, then great strides toward the control of disease would have been made. In particular we wish to focus attention on ribonucleic acid. This substance is widely distributed among microorganisms in the cells, and it often precipitates in the form of inclusions (volutin). Among the bacteria it is usually present in both Gram-positive and Gram-negative species; even bacteria which do not normally deposit volutin may contain ribonucleic acid in a large proportion. There are indications that the ribonucleic acid metabolism is of less importance in the cells of higher plants and animals than in bacteria, so that an agent which *specifically* inhibits the formation of ribonucleic acid would not be of high toxicity, at least to adult animals, and would considerably increase the susceptibility of the bacterial cell to the action of body fluids or of chemotherapeutic agents. Indeed the action of penicillin on bacteria—formation of cells of abnormal morphology, interference with cell division, tendency to render Gram-positive bacteria Gram-negative (for the literature see the section on form and size)—suggests that penicillin may partly act as such an agent. It seems, therefore, that a rational approach to chemotherapy should begin by a study of the metabolism of the principal phosphorus-containing compounds, particularly that of ribonucleic acid.

BIBLIOGRAPHY

ABRAMSON, H. A., MOYER, L. S., and GORIN, M. H. 1942. Electrophoresis of proteins.

ADOLPH, E. F., and BAYNE-JONES, S. 1932. Growth in size of micro-organisms measured from motion pictures. II. *Bacillus megatherium*. Jour. Cell. Comp. Physiol. 1: 409–427.

ALLEN, L. A., APPLEBY, J. C., and WOLF, J. 1939. Cytological appearances in a spore-forming bacillus. Evidence of meiosis. Zentr. Bakt. Parasitenk. II. 100: 3–16.

ALLYN, W. P. and BALDWIN, I. L. 1930. The effect of the oxidation-reduction character of the medium on the growth of an aerobic form of bacteria. Jour. Bact. 20: 417–437.

——— and ———. 1932. Oxidation-reduction potentials in relation to the growth of an aerobic form of bacteria. Jour. Bact. 23: 369–398.

ALTURE-WERBER, E., LIPSCHITZ, R., KASHDAN, F., and ROSENBLATT, P. 1945. The effect of incompletely inhibitory concentrations of penicillin on *Escherichia coli*. Jour. Bact. 50: 291–295

Angerer, R. von 1938. Untersuchungen über die Ursachen den Resistenz von Bazillensporen. Arch. Hyg. Bakt. **121**: 12–55.

Badian, J. 1933. Eine cytologische Untersuchung über das Chromatin und den Entwicklungszyklus der Bakterien. Arch. Mikrobiol. **4**: 409–418.

———. 1935. Sur la cytologie du *Bacillus megatherium*. Acta Soc. Bot. Poloniae **12**: 69–74.

Bartholomew, J. W. 1946. Gram-positive characteristics of *Neisseria*. Jour. Bact. **51**: 584–585.

——— and Umbreit, W. W. 1944. Ribonucleic acid and the Gram stain. Jour. Bact. **48**: 567–578.

Baylor, M. B., Appleman, M. D., Sears, O. H., and Clark, G. L. 1945. Some morphological characteristics of nodule bacteria as shown by the electron microscope. II. Jour. Bact. **50**: 249–256.

Bayne-Jones, S., and Adolph, E. F. 1932*a*. Growth in size of micro-organisms measured from motion pictures. I. Yeast, *Saccharomyces cerevisiae*. Jour. Cell. Comp. Physiol. **1**: 387–407.

——— and ———. 1932*b*. Growth in size of micro-organisms measured from motion pictures. III. *Bacterium coli*. Jour. Cell. Comp. Physiol. **2**: 329–348.

——— and Sandholzer, L. A. 1933. Changes in the shape and size of *Bacterium coli* and *Bacillus megatherium* under the influence of bacteriophage—A motion photo-micrographic analysis of the mechanism of lysis. Jour. Expt. Med. **57**: 279–304.

Bekker, J. H. 1944–45. The antigenic properties of bacterial spores. Antonio von Leeuwenhoek's Jour. Microbiol. Serol. **10**: 67–70.

Boivin, A., and Mesrobeanu, L. 1938*a*. Les antigènes somatiques et flagellaires des bactéries. Ann. Inst. Pasteur **61**: 426–478.

——— and ———. 1938*b*. Sur la résistance à l'acide trichloracétique de l'antigène flagellaire (antigène H) du bacille typhique et sur la nature chimique possible de cet antigène. Compt. Rend. Soc. Biol. (Paris) **129**: 136–138.

Bovarnick, M. 1942. The formation of extracellular d(-) glutamic acid polypeptide by *Bacillus subtilis*. Jour. Biol. Chem. **145**: 415–424.

Bradbury, F. R., and Jordan, D. O. 1942. The surface behavior of antibacterial substances. I. Sulfanilamide and related substances. Biochem. Jour. **36**: 287–293.

Braun, W. 1947. Bacterial dissociation. A critical review of a phenomenon of bacterial variation. Bact. Rev. **11**: 75–114.

Brown, H. P. 1945. On the structure and mechanics of the protozoan flagellum. Ohio Jour. Sci. **45**: 247–278.

Buck, T. C. 1947. Further studies on *Lactobacillus enzymo-thermophilus*. Jour. Bact. **54**: 12.

Burdon, K. L. 1946. Fatty material in bacteria and fungi revealed by staining dried, fixed slide preparations. Jour. Bact. **52**: 665–678.

Cavalli, L. L. 1945. Contributo al problema del nucleo batterico. Osservazione supermicroscopica e analisi biofisica con raggi x in *B. coli*. Atti Ist. Bot. Univ. V. **6**: 3–25.

Chain, E., and Duthie, E. S. 1945. Bactericidal and bacteriolytic action of penicillin on the staphylococcus. Lancet **248**: 652–657.

Chance, H. L. 1938. Mitosis-like activity in *Bacillus* sp. A preliminary report. Jour. Bact. **35**: 347–350.

Christian, M. I. 1931. A contribution to the bacteriology of commercial sterilized milk. Part II. The coconut or carbolic taint. A study of the causal organism and the factors governing its spore-formation. Jour. Dairy Res. **3**: 113–132.

Clark, P. F., and Ruehl, W. H. 1919. Morphologic changes during the growth of bacteria. Jour. Bact. **4**: 615–629.

Cohen, S. S. 1945. The chemical alteration of a bacterial surface with special reference to the agglutination of *B. proteus* OX- 19. Jour. Exp. Med. **82**: 133–142.

Cook, R. P. 1931. Some factors influencing spore formation in *B. subtilis* and the metabolism of its spores. Zentr. Bakt., Parasitenk. I. Orig. **122**: 329–335.

Curran, H. R. 1931. Influence of osmotic pressure upon spore germination. Jour. Bact. **21**: 197–209.

———— Brunsetter, B. C., and Myers, A. T. 1943. Spectrochemical analysis of vegetative cells and spores of bacteria. Jour. Bact. **45**: 485–494.

———— and Evans, F. R. 1937. The importance of enrichments in the cultivation of bacterial spores previously exposed to lethal agencies. Jour. Bact. **34**: 179–189.

and ————. 1945. Heat activation inducing germination in the spores of thermotolerant and thermophilic aerobic bacteria. Jour. Bact. **49**: 335–346.

Delaporte, B. 1934. Sur la structure et le processus de sporulation de l'*Oscillospira Guilliermondi*. Compt. Rend. Acad. Sci. (Paris) **198**: 1187–1189.

————. 1935. Recherches sur la cytologie des bacilles de l'intestin des têtards. Compt. Rend. Acad. Sci. (Paris) **201**: 1409–1411.

————. 1936*a*. Nouvelles recherches sur la cytologie des bactéries. Compt. Rend. Acad. Sci. (Paris) **202**: 1382–1384.

————. 1936*b*. Recherches cytologiques sur le groupe des coccacées. Compt. Rend. Acad. Sci. (Paris) **203**: 199–201.

————. 1939. Sur les acides nucléiques des levures et leur localisation. Rev. Gén. Bot. **51**: 449–482.

1939–40. Recherches cytologiques sur les bactéries et les cyanophycées. Rev. Gén. Bot. **51**: 615–643, 689–708, 748–768; **52**: 112–160.

Dienes, L. 1939. "L" Organisms of Kleineberger and *Streptobacillus moniliformis*. Jour. Inf. Dis. **65**: 24–42.

————. 1940. L type of growth in *Gonococcus* cultures. Proc. Soc. Exp. Biol. Med. **44**: 470–471.

————. 1942. The significance of the large bodies and the development of L type of colonies in bacterial cultures. Jour. Bact. **44**: 37–73.

————. 1943. Reproduction of bacteria from the large bodies of *Streptobacillus moniliformis*. Proc. Soc. Exp. Biol. Med. **53**: 84–86.

————. 1944. L type of growth in cultures of a hemolytic Parainfluenza bacillus. Proc. Soc. Exp. Biol. Med. **55**: 142–144.

————. 1945. Morphology and nature of the pleuropneumonia group of organisms. Jour. Bact. **50**: 441–458.

————. 1946. Complex reproductive processes in bacteria. Cold Spring Harbor Symp. Quant. Biol. **11**: 51–59.

————. 1947. The morphology of the L_1 of Klieneberger and its relationship to *Streptobacillus moniliformis*. Jour. Bact. **54**: 231–237.

and Smith, W. E. 1944. The significance of pleomorphism in *Bacteroides* strains. Jour. Bact. **48**: 125–153.

Dobell, C. C. 1911. Contributions to the cytology of bacteria. Quart. Jour. Micr. Sci. **56**: 395–506.

Dubin, I. N. and Sharp, D. G. 1944. Comparison of the morphology of *Bacillus megatherium* with light and electron microscopy. Jour. Bact. **48**: 313–330.

Dubos, R. J. 1929*a*. Observations on the oxidation-reduction properties of sterile bacteriological media. Jour. Exp. Med. **49**: 507–523.

————. 1929*b*. The initiation of growth of certain facultative anaerobes as related to oxidation-reduction processes in the medium. Jour. Exp. Med. **49**: 559–573.

——————. 1939. Enzymatic analysis of the antigenic structure of pneumococci. Ergeb. Enzymforsch., Leipzig, Akad. Verlagsgesel. M.B.H. 135–148.

——————. 1945. The bacterial cell.

—————— and Avery, O. T. 1931. Decomposition of the capsular polysaccharide of pneumococcus type III by a bacterial enzyme. Jour. Exp. Med. **54**: 51–71.

—————— and MacLeod, C. M. 1938. The effect of a tissue enzyme upon pneumococci. Jour. Exp. Med. **67**: 791–797.

Dufrenoy, J. and Pratt, R. 1947. Cytochemical mechanism of penicillin action. III. Effect on reaction to the Gram stain. Jour. Bact. **54**: 283–289.

Dutky, S. R. 1933. Reported by Knaysi, 1938.

——————. 1947. Preliminary observations on the growth requirements of *Bacillus popilliae* Dutky and *Bacillus lentimorbus* Dutky. Jour. Bact. **54**: 267.

Dyar, M. T. 1947*a*. A cell wall stain employing a cationic surface-active agent as a mordant. Jour. Bact. **53**: 498.

——————. 1947*b*. Studies on the surface lipids of *Micrococcus aureus*. Jour. Bact. **54**: 17.

——————. 1947*c*. Electrophoretic studies on the chemical nature of bacterial surfaces. Doctor's thesis, Cornell Univ.

—————— and Knaysi, G. 1947. Reported in Dyar, 1947*c*.

—————— and Ordal, E. J. 1946. Electrokinetic studies on bacterial surfaces. I. The effects of surface-active agents on the electrophoretic mobilities of bacteria. Jour. Bact. **51**: 149–167.

Effront, J. 1914. Les catalyseurs biochimiques dans la vie et dans l'industrie.

Ellis, D. 1932. Sulphur bacteria.

Enderlein, G. 1925. Bakterien-Cyclogenie.

Esty, J. R. and Williams, C. C. 1920. Resistant bacteria causing spoilage in canned foods. Abst. Bact. **4**: 11.

Etinger-Tulczynska, R. 1933. Bakterienkapseln und Quellungsreaktion. Zeits. Hyg., Infektionskrankheit **114**: 769–789.

Evans, F. R. and Curran, H. R. 1943. The accelerating effect of sublethal heat on spore germination in mesophilic aerobic bacteria. Jour. Bact. **46**: 513–523.

Feulgen, R. and Rossenbeck, H. 1924. Mikroskopischchemischer Nachweis einer Nucleinsäure und die darauf beruhende elektive Färbung von Zellkernen in mikroskopischen Präparaten. Zeits. Physik. Chem. **135**: 203–248.

Fischer, A. 1891. Die Plasmolyse der Bakterien. Ber. Verh. Kgl. Sächs. Ges. Wiss. Leipzig, Math.-Phys. Cl. **43**: 52–74.

Fisher, A. M. 1946. A study on the mechanism of action of penicillin as shown by its effect on bacterial morphology. Jour. Bact. **52**: 539–554.

Fleming, A. 1941. Mode of action of chemotherapeutic agents. Lancet **241**: 761.

Friedman, C. A. and Henry, B. S. 1930. Bound water content of vegetative and spore forms of bacteria. Jour. Bact. **36**: 99–105.

Gardner, A. D. 1940. Morphological effects of penicillin on bacteria. Nature **146**: 837.

Garrod, L. P. 1945. The action of penicillin on bacteria. Brit. Med. Jour. **1**: 107–110.

Gay, F. P. and Clark, A. R. 1937. On the mode of action of sulfanilamide in experimental *Streptococcus* empyema. Jour. Exp. Med. **66**: 535–548.

Gibson, T. and Abdel-Malek, Y. 1945. The formation of carbon dioxide by lactic acid bacteria and *Bacillus licheniformis* and a cultural method of detecting the process. Jour. Dairy Res. **14**: 35–44.

GOWEN, J. W. and LINCOLN, R. E. 1942. A test for sexual fusion in bacteria. Jour. Bact. **44**: 551–554.

GREENE, H. C. 1938. Colony organization of certain bacteria with reference to sporulation. Jour. Bact. **35**: 261–270.

GUTSTEIN, M. 1924. Das Ektoplasma der Bakterien. I. Ueber eine allgemeine Methode zur Darstellung des Ektoplasmas der Grampositiven Bakterien. Zentr. Bakt. Parasitenk. I. Orig. **93**: 393–402.

HADLEY, P. 1928. The Twort-d'Herelle phenomenon. A critical review and presentation of a new conception (homogamic theory) of bacteriophage action. Jour. Inf. Dis. **42**: 263–434.

HANBY, W. E. and RYDON, H. N. 1946. The capsular substance of *Bacillus anthracis*. Biochem. Jour. **40**: 297–307.

HAYWARD, A. E. 1943. Some physiological factors in spore production. Jour. Bact. **45**: 200.

———, MARCHETTA, J. A. and HUTTON, R. S. 1946. Strain variation as a factor in the sporulating properties of the so-called *Bacillus globigii*. Jour. Bact. **52**: 51–54.

HEIDELBERGER, M., KENDALL, F. E. and SCHERP, H. W. 1936. Specific polysaccharides of type I, II, and III pneumococcus; revision of methods and data. Jour. Exp. Med. **64**: 559–572.

HENRICI, A. T. 1928. Morphologic variation and the rate of growth of bacteria.

HENRY, B. S. and FRIEDMAN, C. A. 1937. The water content of bacterial spores. Jour. Bact. **33**: 323–329.

HENRY, H. and STACEY, M. 1943. Histochemistry of the Gram-staining reaction. Nature **151**: 671.

——— and ———. 1946. Histochemistry of the Gram-staining reaction for microorganisms. Proc. Royal Soc., London, B, **133**: 391–406.

HILLIER, J. and KNAYSI, G. 1947*a*. A study with the electron microscope of the origin of flagella in *Bacillus mycoides*. To be published.

——— and ———. 1947*b*. Unpublished.

HOBBY, G. L., and DAWSON, M. H. 1944. Effect of rate of growth of bacteria on action of penicillin. Proc. Soc. Exp. Biol. Med. **56**: 181–184.

———, MEYER, K. and CHAFFEE, E. 1942. Observation on the mechanism of action of penicillin. Proc. Soc. Exp. Biol. Med. **50**: 281–285.

HODGE, H. M. and KNAYSI, G. 1937. *In* Knaysi, 1944.

HOFER, A. W. 1944. Electron microscope studies on *Azotobacter* flagellation and *Rhizobium* bacteriophage. Jour. Bact. **47**: 415–416.

HOOGERHEIDE, J. C. 1939. Studies on capsule formation. I. The condition under which *Klebsiella pneumoniae* forms capsules. Jour. Bact. **38**: 367–388.

———. 1940. Studies on capsule formation. II. The influence of electrolytes on capsule formation by *Klebsiella pneumoniae*. Jour. Bact. **39**: 649–658.

HOWIE, J. W. and CRUICKSHANK, J. 1940. Bacterial spores as antigens. Jour. Path. Bact. **50**: 235–242.

HOYT, A. 1935. Studies upon growth phases of *Clostridium septicum*. Jour. Bact. **30**: 243–251.

HUTCHINSON, W. G., and MCKRACKEN, M. R. 1943. Study of flagella of a fresh water bacterium by motion microphotography and electron micrography. Jour. Bact. **45**: 305.

IMŠENECKI, A. 1940. [Structure of bacteria]. [Soviet Academy of Science]. [In Russian].

———. 1945. On the structure of anaerobic bacteria. Jour. Bact. **49**: 1–5.

IVANOVICS, G. 1937. Unter welchen Bedingungen werden bei der Nährbodenzüchtung der Milzbrandbazillen Kapseln gebildet. Zentr. Bakt. Parasitenk. I. Orig. **138**: 449–455.

———. 1938. Das kulturelle Verhalten des *Bacillus mesentericus vulgatus mucosus,* insbesondere in Bezug auf die Produktion der P-Substanz. Zentr. Bakt. Parasitenk. I. Orig. **142**: 52–64.

———. 1939. Das Schicksal der Kapselsubstanz (P-Substanz) der Milzbrandbazillen im Organismus. Zeits. Immunitätsforsch. **96**: 408–412.

JOHNSON, F. H. 1944. Observations on the electron microscopy of *B. cereus,* and tyrothricin action. Jour. Bact. **47**: 551–557.

——— and DENNISON, W. L. 1944. The volume change accompanying the Quellung reaction of pneumococci. Jour. Immunol. **48**: 317–323.

———, ZWORYKIN, N. and WARREN, G. 1943. A study of luminous bacterial cells and cytolysates with the electron microscope. Jour. Bact. **46**: 167–185.

KAPLAN, I. and WILLIAMS, J. W. 1941. Spore formation among the anaerobic bacteria. I. The formation of spores by *Clostridium sporogenes* in nutrient agar media. Jour. Bact. **42**: 265–282.

KING, R. L. and BEAMS, H. W. 1942. Ultracentrifugation and cytology of *Spirillum volutans.* Jour. Bact. **44**: 597–603.

KLIENEBERGER-NOBEL, E. 1945. Changes in the nuclear structure of bacteria, *tuberculosis.* Jour. Inf. Dis. **45**: 13–33.

KNAYSI, G. 1929. The cytology and microchemistry of *Mycobacterium tuberculosis.* Jour. Inf. Dis. **45**: 13–33.

———. 1930. The cell structure and cell division of *Bacillus subtilis.* Jour. Bact. **19**: 113–115.

———. 1933. Morphological and cultural studies of *Bacillus megatherium* with special reference to dissociation. Jour. Bact. **26**: 623–644.

———. 1935*a*. Further observations on certain variants of *Bacillus megatherium.* Jour. Bact. **29**: 389–390.

———. 1935*b*. The effect of temperature, pH, and salts on the oxidation-reduction potential of sterile meat infusion broth. Unpublished.

———. 1938. Cytology of bacteria. Bot. Rev. **4**: 83–112.

———. 1940. A photomicrographic study of the rate of growth of some yeasts and bacteria. Jour. Bact. **40**: 247–253.

———. 1941*a*. Observations on the cell division of some yeasts and bacteria. Jour. Bact. **41**: 141–150.

———. 1941*b*. A morphological study of *Streptococcus fecalis.* Jour. Bact. **42**: 575–586.

———. 1941*c*. A nucleus-like structure in a *Staphylococcus.* Science **94**: 234.

———. 1942. The demonstration of a nucleus in the cell of a *Staphylococcus.* Jour. Bact. **43**: 365–385.

———. 1943*a*. A cytological and microchemical study of *Thiobacillus thiooxidans.* Jour. Bact. **46**: 451–461.

———. 1943*b*. Unpublished.

———. 1944. Elements of bacterial cytology.

———. 1945*a*. On the origin and fate of the fatty inclusions in a strain of *Bacillus cereus.* Science **102**: 424.

———. 1945*b*. On the microscopic methods of measuring the dimensions of the bacterial cell. Jour. Bact. **49**: 375–381.

———. 1945*c*. A study of some environmental factors which control endospore formation by a strain of *Bacillus mycoides.* Jour. Bact. **49**: 473–493.

———. 1945*d*. Investigation of the existence and nature of reserve material in the endospore of a strain of *Bacillus mycoides* by an indirect method. Jour. Bact. **49**: 617–622.

———. 1946*a*. On the existence, morphology, nature, and functions of the cytoplasmic membrane in the bacterial cell. Jour. Bact. **51**: 113–121.

————. 1946*b*. Further observations on the nuclear material of the bacterial cell. Jour. Bact. **51**: 177–180.

————. 1946*c*. On the process of sporulation in a strain of *Bacillus cereus*. Jour. Bact. **51**: 187–197.

————. 1946*d*. On the inclusions of *Hansenula anomala*. Jour. Bact. **52**: 487–488.

————. 1947*a*. Germination of the endospores of *Bacillus mycoides* in a nitrogen-free medium and some properties of the developed cells. To be published.

————. 1947*b*. Unpublished.

———— and Baker, R. F. 1947*a*. Demonstration, with the electron microscope, of nucleus-like bodies in cells of *Bacillus mycoides* grown in nitrogen-free media. Jour. Appl. Physics **18**: 271–272.

———— and ————. 1947*b*. Demonstration, with the electron microscope, of a nucleus in *Bacillus mycoides* grown in a nitrogen-free medium. Jour. Bact. **53**: 539–553.

————, ———— and Hillier, J. 1947. A study, with the high-voltage electron microscope, of the life cycle and structure of the endospore in *Bacillus mycoides*. Jour. Bact. **53**: 525–537.

———— and Gunsalus, I. C. 1944. A study of the so-called Marburg and the Lawrence and Ford strains of *Bacillus subtilis*. Jour. Bact. **47**: 381–389.

———— and Hillier, J. 1947. Germination of the endospore of *Bacillus megatherium* and the structure of the spore coat as seen with the electron microscope. To be published.

———— and Mudd, S. 1943. The internal structure of certain bacteria as revealed by the electron microscope—a contribution to the study of the bacterial nucleus. Jour. Bact. **45**: 349–359.

Kramár, E. 1921. Untersuchungen über die chemische Beschaffenheit der Kapselsubstanz einiger Kapselbakterien. Zentr. Bakt. Parasitenk. I. Orig. **87**: 401–406.

Krauskopf, E. J. and McCoy, E. 1937. The serology of spores of *Bacillus niger* with special reference to the H antigen. Jour. Inf. Dis. **61**: 251–256.

Lamanna, C. 1937. The role of the coat in the resistance to moist heat of bacterial endospores as indicated by modes of germination. Master's Thesis, Cornell Univ.

————. 1940*a*. The taxonomy of the genus *Bacillus*. Modes of spore germination. Jour. Bact. **40**: 347–360.

————. 1940*b*. The taxonomy of the genus *Bacillus*. II. Differentiation of small celled species by means of spore antigens. Jour. Inf. Dis. **67**: 193–204.

————. 1940*c*. The taxonomy of the genus *Bacillus*. III. Differentiation of the large celled species by means of spore antigens. Jour. Inf. Dis. **67**: 205–212.

————. 1942*a*. Relation of maximum growth temperature to resistance to heat. Jour. Bact. **44**: 29–35.

————. 1942*b*. The status of *Bacillus subtilis*, including a note on the separation of precipitinogens from bacterial spores. Jour. Bact. **44**: 611–617.

————. 1946. The nature of the acid-fast stain. Jour. Bact. **52**: 99–103.
1947. Personal Communication.

Lasseur, P., Dupaix, A. and Georges, L. 1932. Influence des électrolytes sur les variations de volume des corps microbiens determinées par le sulfocyanure de potassium. Trav. Labor. Microbiol. Faculté Pharm. Nancy. Fasc. 5, 99–104.

Lederberg, J., and Tatum, E. L. 1946. Gene recombination in *Escherichia coli*. Nature **158**: 558.

Lee, S. W., Foley, E. J. and Epstein, J. A. 1944. Mode of action of penicillin. I. Bacterial growth and penicillin activity-*Staphylococcus aureus* F.D.A. Jour. Bact. **48**: 393–399.

Lewis, I. M. 1941. The cytology of bacteria. Bact. Rev. **5**: 181–230.

————. 1942. The cytology of bacteria. Chron. Bot. **7**: 249–250.

Lockwood, J. S. 1938. Studies on the mechanism of action of sulfanilamide. The effect of sulfanilamide in serum and blood on hemolytic streptococci *in vitro*. Jour. Immunol. **35**: 155–193.

Lofgren, R. and Soule, M. H. 1945. The structure of *Spirochaeta novyi* as revealed by the electron microscope. Jour. Bact. **50**: 679–690.

Löhnis, F. 1921. Studies upon the life cycles of the bacteria. Nat. Acad. Sci., Mem. **16** (*2d* Mem.) 252 pp.

Lominsky, I. and Lendrum, A. C. 1942. The effect of surface-active agents on *B. proteus*. Jour. Pathol. Bact. **54**: 421–433.

Lowndes, A. G. 1941. On flagellar movement in unicellular organisms. Proc. Zool. Soc. London **111A**: 111–134.

Luria, S. E. 1947. Recent advances in bacterial genetics. Bact. Rev. **11**: 1–40.

Macierewicz-Straczyńska. 1937. Dziatania Temperatury i pH na Kielkowanie Spor Bakterii. (Wirkung von Temperatur und pH auf die Keimung von Bakteriensporen). Acta Soc. Bot., Poloniae **14**: 371–408.

Malek, I. 1938. Sur la présence de l'antigène Vi dans les différentes souches *d'Eberthella typhi*. II. Influence de l'antigène H *d'Eberthella typhi* sur la flocculation par les ions H. III. Influence de l'antigène Vi sur la flocculation *d'Eberthella typhi* par les ions H. IV. Influence de l'antigène O sur la flocculation *d'Eberthella typhi* par les ions H. V. Les propriétés antigéniques de la souch « R O » et ses relations avec les souches O et H *d'Eberthella typhi*. Compt. Rend. Soc. Biol. **129**: 785–788, 795–804.

McClean, D. 1941. The capsulation of streptococci and its relation to diffusion factor (hyaluronidase). Jour. Pathol. Bact. **53**: 13–27, 156–158.

————. 1942. The *in-vivo* decapsulation of streptococci by hyaluronidase. Jour. Pathol. Bact. **54**: 284–286.

Mellon, R. R. 1925. Studies in microbic heredity. I. Observations on a primitive form of sexuality (zygospore formation) in the colon-typhoid group Jour. Bact. **10**: 481–511.

Metzner, P. 1920. Zur Mechanik der Geisselbewegung. Biol. Zentr. **40**: 49–87.

Miller, C. P. and Foster, A. Z. 1944. Studies on the action of penicillin. III. Bactericidal action of penicillin on meningococcus *in vitro*. Proc. Soc. Exp. Biol. Med. **56**: 205–208.

————, Scott, W. W. and Moeller, V. 1944. Studies on the action of penicillin. The rapidity of its therapeutic effect on gonococcic urethritis. Jour. Am. Med. Assoc. **125**: 607–610.

Morgan, H. R. and Beckwith, T. D. 1939. Mucoid dissociation in the colon-typhoid-salmonella group. Jour. Inf. Dis. **65**: 113–124.

Morrison, E. W. and Rettger, L. F. 1930. Bacterial spores. II. A study of bacterial spore germination in relation to environment. Jour. Bact. **20**: 313–342.

Morton, H. E. and Anderson, T. F. 1942. Observations on the morphology of *Leptospira* and the Nichols' strain of *Treponema pallidum* with the aid of the RCA electron microscope. Jour. Bact. **43**: 64–65.

Moyer, L. S. 1936. Changes in electrokinetic potential of bacteria at various phases of the culture cycle. Jour. Bact. **32**: 433–464.

Mudd, S. 1944. Pathogenic bacteria, rikettsias and viruses as shown by the electron microscope. II. Relationships to immunity. Jour. Am. Med. Assoc. **126**: 632–639.

——— and Anderson, T. F. 1941. Demonstration by the electron microscope of the combination of antibodies with flagellar and somatic antigens. Jour. Immunol. **42**: 251–266.

——— and ———. 1944. Pathogenic bacteria, rickettsias, and viruses as shown by the electron microscope. I. Morphology. Jour. Am. Med. Assoc. **126**: 561–571.

———, Heinmets, F. and Anderson, T. F. 1943*a*. Bacterial morphology as shown by the electron microscope. VI. Capsule, cell-wall and inner protoplasm of pneumococcus type III. Jour. Bact. **46**: 205–211.

———, ——— and ———. 1943*b*. The pneumococcal swelling reaction, studied with the aid of the electron microscope. Jour. Exp. Med., **78**: 327–332.

———, Polevitzky, K. and Anderson, T. F. 1943. Bacterial morphology as shown by the electron microscope. V. *Treponema pallidum*, T. *macrodentium* and T. *microdentium*. Jour. Bact. **46**: 15–24.

———, ———, ——— and Chambers, L. A. 1941. Bacterial morphology as shown by the electron microscope. II. The bacterial cell-wall in the genus *Bacillus*. Jour. Bact. **42**: 251–264.

Nelson, F. E. 1944. Factors which influence the growth of heat-treated bacteria. II. Further studies on media. Jour. Bact. **48**: 473–477.

Neri, F. 1940. Beobachtungen über den Geisselapparat der Bakterien (mit einer neuen Färbungsmethode der Bakteriengeisseln). Zentr. Bakt. Parasitenk. I. Orig. **146**: 166–176.

Ógiuti, K. 1936. Untersuchungen über die Geschwindigkeit der Eigenbewegung von Bakterien. Jap. Jour. Exp. Med. **14**: 19–28.

Ogonuki, H. and Miyata, T. 1940. The relationship between the acid agglutination in *Salmonella* group. Kitasato Arch. Exp. Med. **17**: 80–87.

Peshkov, M. A. 1945. The cytology and karyology of the colon-typhoid group. Am. Rev. Soviet Med. **2**: 342–348.

Piekarski, G. 1938. Zytologische Untersuchungen an Bakterien in ultravioletten Licht. Zentr. Bakt., Parasitenk. I. Orig. **142**: 69–79.

———. 1939*a*. Lichtoptische und übermikroskopische Untersuchungen zum Problem des Bakterienzellkerns. Bericht über die I. Grossdeutsche Tagung der "Deutschen Vereinigung für Mikrobiologie". *In* Zentr. Bakt. Parasitenk. I. Orig. **144**: 140–147.

———. 1939*b*. Zum Problem des Bakterienzellkerns. 3d Int. Congr. Microbiol., N. Y. Abstracts of Communications, 46–47.

———. 1940. Ueber kernähnliche Strukturen bei *Bacillus mycoides* Flügge. Arch. Mikrobiol. **11**: 406–431.

and Ruska, H. 1939. Übermikroskopische Untersuchungen an Bakterien unter besonderer Berücksichtigung der sogenannten Nucleoide. Arch. Mikrobiol. **10**: 302–321.

Pijper, A. 1938. Dark-ground studies of flagellar and somatic agglutination of *B. typhosus*. Jour. Pathol. Bact. **47**: 1–17.

———. 1941*a*. Dark-ground studies of Vi agglutination of *B. typhosus*. Jour. Pathol. Bact. **53**: 431–436.

———. 1941*b*. Microcinematography of the agglutination of typhoid bacilli. Jour. Bact. **42**: 395–409.

———. 1946. Shape and motility of bacteria. Jour. Pathol. Bact. **58**: 325–342.

———. 1947*a*. Methylcellulose and bacterial motility. Jour. Bact. **53**: 257–269.

1947*b*. Filming as a method of research in microbiology. *In* Antonie van Leeuwenhoek **12**: 26–32.

Porter, K. R. and Yegian, D. 1945. Some artifacts encountered in stained preparations of tubercle bacilli. II. Much granules and beads. Jour. Bact. **50**: 563–575.

PRATT, R. and DUFRENOY, J. 1947. Cytochemical mechanisms of penicillin action. II. Changes in reactions of *Staphylococcus aureus* to vital dyes. Jour. Bact. 54: 127–133.

RANTZ, L. A. and KIRBY, W. M. M. 1944. The action of penicillin on the staphylococcus *in vitro*. Jour. Immunol. 48: 335–343.

REEVES, R. E. and ANDERSON, R. J. 1937. The chemistry of the lipides of tubercle bacilli. XLVII. The composition of the avian tubercle bacillus wax. Jour. Am. Chem. Soc. 59: 858–861.

ROBERTS, J. L. 1942. Cytological changes occurring in *Clostridium pasteurianum* during spore formation Jour. Bact. 43: 777.

———— and BALDWIN, I. L. 1942. Spore formation by *Bacillus subtilis* in peptone solutions altered by treatment with activated charcoal. Jour. Bact. 44: 653–659.

ROBINOW, C. F. 1942. A study of the nuclear apparatus of bacteria. Proc. Royal Soc. London, B, 130: 299–324.

————. 1944. Cytological observations on *Bact. coli, Proteus vulgaris* and various aerobic sporeforming bacteria with special reference to the nuclear structures. Jour. Hyg. Cambridge 43: 413–423.

1945. Nuclear apparatus and cell structure of rod-shaped bacteria. *In* The Bacterial Cell, by R. J. Dubos, 353–377.

RUEHLE, G. L. A. 1923. The enzyme content of bacterial spores. Jour. Bact. 8: 487–491.

SCHAUDINN, F. 1902. Beiträge zur Kenntnis der Bakterien und verwandter Organismen. I. *Bacillus bütschlii* n. sp. Arch. Parasitenk. 1: 306–343.

————. 1903. Beiträge zur Kenntnis der Bakterien und verwandter Organismen. II. *Bacillus sporonema* n. sp. Arch. Protistenk. 2: 416–444.

SEVAG, M. G., SMOLENS, J. and LACKMAN, D. B. 1940. The nucleic acid content and distribution in *Streptococcus pyogenes*. Jour. Biol. Chem. 134: 523–529.

SHANAHAN, A. J., EISENSTARK, A. and TANNER, F. W. 1947. Morphology of *Escherichia coli* exposed to penicillin as observed with the electron microscope. Jour. Bact. 54: 183–189.

SHAW, M. 1937. Decomposition of pneumococcus carbohydrate by the combined activity of strains of two bacterial species. Jour. Bact. 33: 644–645.

SHERMAN, J. M. and WING, H. U. 1937. Attempts to reveal sex in bacteria; with some light on fermentative variability in the coli-aerogenes group. Jour. Bact. 33: 315–321.

SICKLES, G. M. and SHAW, M. 1933. Microorganisms which decompose the specific carbohydrate of pneumococcus types II and III. Jour. Inf. Dis. 53: 38–43.

SMITH, L. D. and HAY, T. 1942. Effect of penicillin on the growth and morphology of *Staphylococcus aureus*. Jour. Franklin Inst. 233: 598–602.

SMITH, W. E., HILLIER, J. and MUDD, S. 1946. Electron microscope studies of organisms of the pleuropneumonia group. Jour. Bact. 51: 583–584.

STANIER, R. Y. 1942. A note on elasticotaxis in myxobacteria. Jour. Bact. 44: 405–412.

STAPP, C. and ZYCHA, H. 1931. Morphologische Untersuchugen an *Bacillus mycoides*; ein Beitrag zur Frage des Pleomorphismus der Bakterien. Arch. Mikrobiol. 2: 493–536.

STARKEY, R. L. 1946. Lipid production by a soil yeast. Jour. Bact. 51: 33–50.

STEARNS, T. W. and ROEPKE, M. H. 1941*a*. Electrophoresis studies on *Brucella*. Jour. Bact. 42: 411–430.

———— and ————. 1941*b*. The effect of dissociation on the electrophoretic mobility of *Brucella*. Jour. Bact. **42**: 745–755.

Stille, B. 1937. Zytologische Untersuchungen an Bakterien mit Hilfe der Feulgenschen Nuclealreaktion. Arch. Mikrobiol. **8**: 125–148.

Stodola, F. H., Lesuk, A. and Anderson, R. J. 1938. The chemistry of the lipids of tubercle bacilli. LIV. The isolation and properties of mycolic acid. Jour. Biol. Chem. **126**: 505–513.

Tarr, H. L. A. 1933. Some observations on the respiratory catalysts present in the spores and vegetative cells of certain aerobic bacilli. Biochem. Jour. **27**: 136–145.

Tatum, E. L. and Lederberg, J. 1947. Gene recombination in the bacterium *Escherichia coli*. Jour. Bact. **53**: 673–684.

Theophilus, D. R. 1937. Influence of growth temperature on the thermal resistance of some aerobic spore forming bacteria from evaporated milk. Panegyric B. W. Hammer. College Press, Inc., Ames, Iowa.

Thomas, A. R., Jr. and Levine, M. 1945. Some effects of penicillin on intestinal bacteria. Jour. Bact. **49**: 623–627.

Todd, E. W. 1945. Bacteriolytic action of penicillin. Lancet **248**: 74–78.

Tunnicliff, R. 1939. The action of prontosil-soluble and sulfanilamide on the phagocytic activity of leukocytes and on the dissociation of streptococci. Jour. Inf. Dis. **64**: 59–65.

Ullrich, H. 1926. Über die Bewegungen von *Beggiatoa mirabilis* und *Oscillatoria Jenensis*. I. Planta **2**: 295–324.

Vendrely, R. and Lipardy, J. 1946. Acides nucléiques et noyaux bacteriens. Compt. Rend. Acad. Sci. (Paris) **223**: 342–344.

Virtanen, A. I. and Pulkki, L. 1933. Biochemische Untersuchungen über Bakteriensporen. Arch. Mikrobiol. **4**: 99–122.

Wámoscher, L. 1930. Versuche über die Struktur der Bakterienzelle. Zeits. Hyg. Infektionsk. **111**: 422–460.

Weiss, L. J. 1943. Electron micrographs of bacteria medicated with penicillin. Proc. Indiana Acad. Sci. **52**: 27–29.

Wile, U. J. and Kearney, E. B. 1943. The morphology of *Treponema pallidum* in the electron microscope: Demonstration of flagella. Jour. Am. Med. Assoc. **122**: 167–168.

————, Picard, R. G. and Kearney, E. B. 1942. The morphology of *Spirochaeta pallida* in the electron microscope. Jour. Am. Med. Assoc. **119**: 880–881.

Wood, W. B., Wood, M. L. and Baldwin, I. L. 1935. The relation of oxidation-reduction potential to the growth of an aerobic microorganism. Jour. Bact. **30**: 593–602.

Wurmser, R. 1930. Oxydations et réductions.

Yegian, D. and Baisden, L. 1942. Factors affecting the beading of the tubercle bacillus stained by the Ziehl-Neelsen technique. Jour. Bact. **44**: 667–672.

———— and Porter, K. R. 1944. Some artifacts encountered in stained preparations of tubercle bacilli. I. Non-acid-fast forms arising from mechanical treatment. Jour. Bact. **48**: 83–91.

TRIARCH BOTANICAL PRODUCTS

Fine Microscope Slides for Critical Botanists

* * * * *

Nothing can take the place of a good microscope slide in botany instruction, especially to show clearly that structure is a basis for function. However, the slide must be *good,* even though it is not perfect. If the slide can be beautiful as well as clear, it becomes a fine piece of teaching equipment.

TRIARCH slides, we believe, embody these points more completely than any other slides on the market, as we stain them regularly with our famous quadruple combination of safranin, crystal violet, fast green and gold orange, unexcelled for tissue differentiation. Also, as we stain all sections by the same schedule, our slides give you comparative checks on age and development of tissues. Many cytological points are emphasized by Triarch stain, and host-parasite relations are clearly demonstrated in pathological tissues.

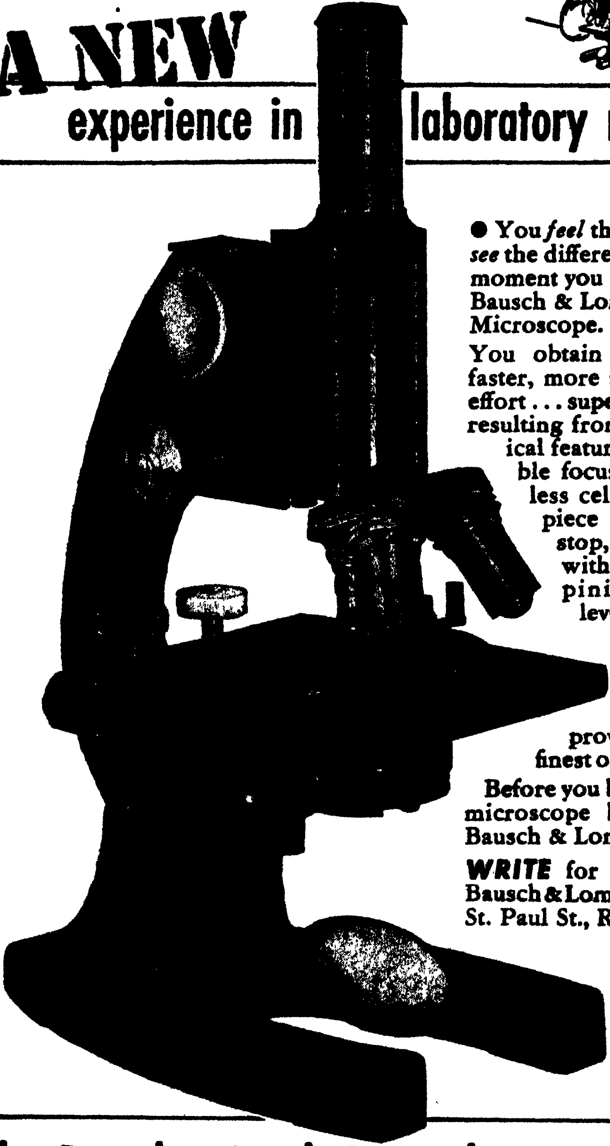
Increasing numbers of botanists are realizing the teaching value of Triarch slides, together with the convenience of Triarch accuracy and prompt service, as evidenced by the fact that we are now shipping out more than 10,000 slides per month. Over 650 colleges and universities in 19 different countries are now counted among our customers.

If you are not already one of our patrons, send for our current catalog, No. 7, which lists over 350 regular catalog items, heavily stocked, together with many supplementary structures for special orders.

* * * * *

Geo. H. Conant

Triarch Botanical Products :-: *Ripon, Wisconsin*



A NEW experience in laboratory microscopy

● You *feel* the difference . . . You *see* the difference . . . from the first moment you begin to focus a new Bausch & Lomb BAV Laboratory Microscope.

You obtain proper adjustment faster, more accurately, with less effort . . . superlative performance resulting from advanced mechanical features such as the variable focus condenser, threadless cell lens mounts, nose piece with roller bearing stop, coarse adjustment with *matched* rack and pinion, and patented lever fine adjustment.

Most important, you *see* better. Images of unmatched clarity and sharpness are provided by the world's finest optical system.

Before you buy a new laboratory microscope be sure to see the Bausch & Lomb BAV.

WRITE for a demonstration to Bausch & Lomb Optical Co., 708-B St. Paul St., Rochester 2, N. Y.

the Bausch & Lomb BAV Laboratory Microscope

Outstanding McGraw-Hill Books

HORMONES AND HORTICULTURE. The Use of Special Chemicals in the Control of Plant Growth

By George S. Avery, Jr., Brooklyn Botanic Garden; and Elizabeth B. Johnson, formerly of Connecticut College. *McGraw-Hill Publications in the Botanical Sciences.* 326 pages, $4.50

The important new applications of special chemicals to the control of plant growth are fully outlined in this timely manual. Application of the growth-controlling hormones to cuttings, fruit production, pre-harvest crop control, production of new varieties, seedless fruit production, and many other growth phenomena are discussed in detail, and popular misconceptions regarding hormone use in the control of plant growth are clarified.

INTRODUCTION TO PALEOBOTANY

By Chester A. Arnold, University of Michigan. *McGraw-Hill Publications in the Botanical Sciences.* 427 pages, $5.50

Meets the urgent need for an up-to-date, comprehensive text on the subject. The approach is botanical rather than geological, and emphasis is placed upon those fossil plants found in the New World. Relation and classification of ancient plants to the major categories are stressed throughout, and the influence of paleobotanical discoveries upon classification is emphasized.

FOREST PATHOLOGY. New 2nd edition

By John S. Boyce, Department of Forestry, Yale University. 526 pages, $6.00

This successful book has been thoroughly revised. Much of it has been entirely rewritten to include a wealth of information on such topics as salt spray injury, Phymatotrichum root rot, phloem necrosis of elm, Adelopus needle cast of Douglas fir, septoria canker of poplars, canker stain of planetree, oak wilt, mimosa wilt, persimmon wilt, and several other rots. New material has been included on the prevention and control of the deterioration of wood in service.

FLOWERS AND FLOWERING PLANTS

By Raymond J. Pool, University of Nebraska. *McGraw-Hill Publications in the Botanical Sciences.* Second edition. 428 pages, $4.50

An introduction to the nature and work of flowers and the classification of flowering plants. The second edition contains a new chapter describing the more common characteristics of roots, stems, and leaves—all fully illustrated.

Send for copies on approval

McGRAW-HILL BOOK COMPANY, Inc.

330 West 42nd Street **New York 18, N. Y.**

THE BOTANICAL REVIEW

VOL. XV MARCH, 1949 No. 3

94
~~53~~
(41)

GERMINATION INHIBITORS

MICHAEL EVENARI
Hebrew University, Jerusalem

INTRODUCTION

Ontogeny is not a continuous process of development, but is interspersed with periods during which development is completely or partially inhibited. The egg cell and the endosperm nucleus before fertilization, the embryo in most ripe seeds, and winter and summer rest periods of trees and shrubs are good examples of such inhibition phases belonging to the normal developmental cycle. Sometimes such inhibitions are brought about by environmental conditions, more often by conditions within the plant itself.

The causes of these inhibitions are manifold. One of them—the one dealt with in this paper—lies in the production of substances inhibiting particular physiological processes which are indispensable for normal development. These inhibitors are very interesting because:

(*a*) They are equally as important in the ontogeny of plants as are growth hormones and other stimulating substances.

(*b*) When excreted they inhibit the development of other species in their surroundings; *Agrostemma Githago,* for instance, cannot germinate in fields of *Beta* because *Beta* excretes a substance which inhibits the germination of *Agrostemma* seeds. Such effects show a striking similarity with the natural function of antibiotics excreted by soil fungi or bacteria which inhibit or destroy other soil organisms in the same locality.

(*c*) They present a problem of the widest physiological significance. Apparently all physiological reactions are governed by a set of "positive" as well as by one of "negative" catalysts whose function it is to delay or inhibit reactions. This was clearly seen as far back as 1903 when Czapek (30) called these substances "negative catalysts" or "antiferments".

This paper deals only with excretory germination inhibitors. All other reasons for inhibition of germination and for primary or secondary (induced) dormancy—*e.g.*, rudimentary embryos, mechanical resistance to expansion of the embryo, seed coats impermeable to water (29, 140)—are excluded.

Germination inhibitors are substances produced by plants or substances of related structure not found in plants which inhibit or delay the germination of seeds of the same or other species. This definition is purely phenomenological, since a better definition, for lack of appropriate knowledge, and based on the inhibitory mechanism, is not possible today. One weak spot in this definition is that we are not sure, as will be seen later, whether germination itself or merely the first phase of subsequent growth is inhibited. If only growth is inhibited, the whole problem would merge into the problem of growth inhibitors. For the time being, then, we may speak of germination inhibitors as substances which from observation have been found to inhibit germination.

TEST METHODS

The prevalent method for testing germination inhibitors is to germinate various seeds in Petri dishes on filter paper moistened by the solutions to be tested (79, 80, 135, 136). Sometimes the parts containing the inhibitors are put into the same dishes with the seeds (148). For the tests different seeds have been used, *e.g.*, wheat (79, 80), *Lepidium sativum* (38, 74), *Papaver orientale* and *Avena sativa* (38). Although many seeds, *e.g.*, *Lepidium sativum*, are more sensitive to inhibitors than wheat grains, the use of wheat is to be recommended, since pure standard varieties of the same quality, which germinate 91% to 100%, are easily obtainable. Oat grains, for which the same holds true are not so well suited, since their glumes already contain an inhibitor. Experiments by the present author to establish a test by using pollen grains as test objects have failed, despite the fact that germination of them is inhibited by all germination inhibitors so far tested (82). Although they are much more sensitive to inhibitors than seeds, their use in tests is impractical because it is nearly impossible to obtain good uniform material the whole year round and it is not easy to create conditions under which their germination invariably reaches 90% to 100% in the controls.

A different test method was used by Froeschel (45–47). He put the materials to be tested for inhibitory content in front of the test seeds. The water supply was thus unilateral, having to pass the materials to be tested in order to reach the seeds. This method is excellent to demonstrate the inhibiting effect but cannot be employed when the relation between inhibitor concentration and inhibition is to be established.

When the effect of inhibition on growth is to be tested, fresh or dry weight of the seedlings or radicles (79, 80) or the length of the radicles (80) is determined in a test and control experiment.

DISTRIBUTION AND LOCALISATION

A perusal of Table 1 shows that germination inhibitors are found in the most different families of the plant kingdom. Their presence seems to be a widely spread phenomenon which is not restricted to phanerogamous plants. Spores and gemmae of cryptogams show an inhibited germination very similar to that of tomato seeds inside the fruit. The gemmae of *Marchantia* do not sprout inside their cupules, and spores of *Funaria hygrometrica* do not germinate within their sporogonia (111). Inhibitors are not confined to seeds or fruit but occur in various parts of plants according to the species:

Fruit

Pulp: pear, apple, *Poterium spinosum, etc.*

Juice: Tomato, *Lonicera, etc.*

Coat: *Triticum, Helianthus, Fagopyrum, etc.*

Seeds

Coat: cabbage, lettuce, *etc.*

Embryo: *Helianthus.* Here a strong inhibition is exerted by all parts of the embryo when they are prepared from non-soaked seeds. When taken out of soaked seeds only the cotyledon inhibits; the radicle inhibits at first but later stimulates; the plumule stimulates (123).

Endosperm: *Iris* (117).

Leaf sap: *Phragmites,* spinach, *etc.* The inhibiting effect of leaf sap was overlooked for a long time (80), although Magnus (97) long ago reported the germination inhibition exerted by the leaf sap of *Phacelia* and *Pelargonium.*

Bulbs: onion, garlic.

Roots: carrot, horseradish, radish, *etc.*

TABLE 1

List of Known Plant-Produced Germination-Inhibitors

Species producing inhibitor	Part of plant containing inhibitor	Authors by whom inhibition was first observed
Agrostemma Githago		13
Allium Cepa	bulb juice	Orig.
A. sativum	" "	Orig.
Ammi Visnaga	fruit	Orig.
Amygdalus communis	seeds	148
Anona sp.	leave sap	80
Apium graveolens	seeds	135
Arachis hypogea	leave sap	80
Armoracia rusticana	" "	80
	root sap	Orig.
A. sativa	fruit	123, 135
Beta saccharifera	fruit coat	47, 135, 136, 144
B. vulgaris (beet root)	leave sap	80
	fruit (all parts)	38, 45, 46, 47
B. vulgaris (mangel)	leave sap	80
B. vulgaris (red beet)	" "	80
Brassica caulocarpa	" "	80
	tuber sap	Orig.
B. nigra	seeds	Orig.
B. oleracea var. *capitata*	leave sap	80
	seed coat	27, 28
Capsicum annuum	fruit	111
	fruit juice	80
C. frutescens var. *cerasiforme*	leave sap	80
Carica Papaya	fruit juice	112
Chenopodium ambrosioides	fruit	148
Cicer arietinum	seeds	164
Citrus Aurantium (*C. sinensis*)	fruit juice	138, 139, Orig.
C. Limonia	" "	138, 139, Orig.
C. maxima	" "	Orig.
C. nobilis var. *deliciosa*	" "	138, 139
Coffea arabica	seeds	148
Coriandrum sativum	fruit	148
Cucumis Melo	fruit juice	135, 139
C. Melo var. *cantaloupensis*	" "	135
C. sativus	leave sap	80
	fruit juice	71
Cucurbita Pepo	seeds	71
	seed coat	71
	embryo	71
	fruit juice	139
Cynanchum acutum	leave sap	80
Cyperus Papyrus	" "	80
Daucus carota var. *sativa*	" "	80
	root sap	Orig.
Ecballium elaterium	fruit juice	Orig.
Ephedra campylopoda	fruit	133
Eryobotrya japonica	leave sap	80

TABLE I—(*Continued*)

LIST OF KNOWN PLANT-PRODUCED GERMINATION-INHIBITORS

Species producing inhibitor	Part of plant containing inhibitor	Authors by whom inhibition was first observed
Erucaria boveana	fruit coat	133
Fagopyrum esculentum	" "	91
Feijowa Sellowiana	leave sap	80
Ficus carica	fruit flesh	74
Foeniculum vulgare	fruit	148
Helianthus annuus	leave sap	80
	fruit coat, seed	123
Hirschfeldia incana	fruit coat	165
Hordeum vulgare	fruit	83, 135
Ipomoea Batatas	leave sap	80
Iris	endosperm	117
Juniperus communis	fruit	148
Lactuca sativa	seeds	125, 126, 135
Lagenaria vulgaris	fruit flesh	110, 111
Lippia nodiflora	leave sap	80
Lonicera tatarica	fruit juice	111
Lonicera xylosteum	fruit juice	111
Lupinus mutabilis	seeds	148
Lycopus europaeus	leave sap	80
Maclura aurantiaca	fruit	111
Mangifera indica	leave sap	80
Matthiola bicornis	fruit coat	133
Nicotiana rustica	leaves	111
	fruit coat	111
Onobrychis cristagalli	pericarp	165
Papaver somniferum	fruit	111
Pelargonium	leaves	97
Persea americana	leave sap	80
Petroselinum hortense (*sativum*)	" "	80
Phacelia tanacetifolia	seeds	97, 114
	leaves	97
Phaseolus vulgaris	leave sap	80
Phragmites communis	" "	80
Phytolacca dioica	fruit	111
Pimpinella anisum	"	148
Piper nigrum	"	148
Pirus communis	fruit flesh	74
Pirus malus	" "	74
	exhalation	75
	endosperm	151
Pisum	seeds	73
Polygonum lapathifolium	leave sap	80
Poterium spinosum	fruit	149
Prunus armeniaca	fruit juice	127
Psidium guajava	leave sap	80
Punica granatum	" "	80
Raphanus sativus	root sap	Orig.
Rumex acetosa	" "	80
Silene Coeli-rosa	seeds (?)	13

TABLE I—(*Continued*)

LIST OF KNOWN PLANT-PRODUCED GERMINATION-INHIBITORS

Species producing inhibitor	Part of plant containing inhibitor	Authors by whom inhibition was first observed
Sinapis alba	fruit coat	133, 165
	pedicels	133
	seeds	Orig.
Sinapis arvensis	fruit coat	148
Solanum coagulans	fruit juice	Orig.
S. lycopersicum	" "	74, 75, 78, 79, 110, 111, 112, 118
	seeds	118
S. Melongena	leave sap	80
Sorbus aucuparia	fruit juice	82
Spinacia oleracea	leave sap	80
	seeds	135
Theobroma Cacao	"	148
Trigonella foenum graecum	"	148
Trifolium pratense	"	45, 46
Triticum sativum	fruit	107
Typha angustata	leave sap	80
Vaccaria pyramidata	seeds	13, 14
Vicia	"	73
Viola tricolor	"	148
Viscum album	leaves	52, 161, 162
	fruit	
Vithania somnifera	fruit juice	Orig.
Vitis vinifera	" "	Orig.
Zea Mays	leave sap	80

SPECIFICITY

Germination inhibitors are non-specific. The inhibitor in seeds of *Trifolium pratense* and fruits of *Beta vulgaris* inhibited the germination of seeds in 28 species belonging to 14 families (45–47), and tomato juice is effective on tomato seeds, wheat, barley, oat and maize grains (78, 79), and on seeds of *Lepidium, Sinapis, Trifolium,* (Orig.), carrot, lettuce, cabbage, sunflower (112), *etc.* The sensitivity of different seeds to the same concentration of inhibitors varies greatly, however. Tomato juice in a dilution of 1 : 25 wholly inhibits the germination of *Lepidium* seeds, whereas wheat grains start to germinate in a dilution of 1 : 4 (Orig.). One-to-four concentration of *Beta* fruit extracts inhibits the germination of seeds of *Amaranthus caudatus* completely, depresses the germination index[1] of *Lepidium* seeds to 4%, but does not affect the germination of *Ipomoea purpurea* (45, 46).

The same seeds react differently to inhibitors of different sources. The fruit juice of *Solanum coagulans,* for instance, inhibits the germination of wheat up to a dilution of 1 : 64, and the last traces of the inhibiting influence disappear only in a dilution of 1 : 500 (Orig.). The corresponding data for tomato juice are: 1 : 4 and 1 : 10, respectively (Orig.); for leaf sap of *Phragmites communis,* 1 : 2 to 1 : 10 and 1 : 50 (76); for garlic juice, 1 : 16 and 1 : 100.

INHIBITION AND OSMOTIC PRESSURE

Oppenheimer (110, 111) has discussed whether osmotic pressure of tomato juice is responsible for its inhibitory action. He came to a negative conclusion, but other authors (38, 89, 93, 99) explain the inhibition solely by the osmotic pressure of the solutions or extracts used. It is certain that in many cases osmotic pressure is one of the inhibiting factors, sometimes the most important, but there are others also.

In grape juice, where the very high (35–37 atm.) osmotic pressure is the main inhibiting agent, there is one of those other factors. In one of our experiments we measured an osmotic pressure of 35.5 atm. caused by a glucose content of 19.4%. This juice completely inhibited down to a dilution of 1 : 8 (4.4 atm.). In a dilution of 1 : 16 (2.1 atm.) the g.i. was 47%. An osmotic pressure of 4.4 atm. has no inhibitory effect or a very slight one, and an osmotic pressure of 2.1 atm. is wholly ineffective on wheat grains. When an artificial grape juice was prepared isotonic to the natural one (19.4% glucose solution), a g.i. of 5.3% was found for the 1 : 4 dilution, of 68% for the 1 : 8 dilution, and of 100.5% for the 1 : 16 dilution (Orig.). All this goes to show that aside from high osmotic pressure there is an additional inhibiting factor present.

There is also other proof to the same end. By yeast fermentation and subsequent removal of the alcohol produced, the sugar content of the juice is reduced to nil (osmotic pressure less than 0.5 atm.). This juice still inhibits completely (1 : 2 dilution gives g.i. of 17%). An isotonic glucose solution treated the same way shows a g.i. of 60% (1: 2 dilution gives a g.i. of 89%), proving that the yeast itself does not produce an inhibitor in sufficient quantity to explain the complete inhibition of the juice (Orig.).

---

[1] Germination index, g.i., expressed as a percentage, is the ratio of the number of seeds germinating under the influence of an inhibitor to the number out of an equal quantity germinating in a pure water control.

In sugar-beet seed extracts, where Duym and co-workers (38) state that the osmotic effect, caused by the inorganic salt constituents of the extract, is the most important factor in causing inhibition, this is true only for "decolorized" extract. In untreated extract another volatile inhibiting factor is present.

For tomato juice and other cases the facts showing that osmotic pressure is only slightly involved or of no importance whatever may be summed up as follows:

(*a*) When grains are germinated in different sugar and salt solutions (KNO_3, KCl, NaCl) isotonic to tomato juice, a slight delaying action on germination is observed, but never inhibition to the extent of that brought about by the tomato juice itself. In one case the g.i. in undiluted tomato juice was zero on the fourth day but 92% to 98% in different isotonic sugar and salt solutions (79).

(*b*) Even at a dilution of 1: 5, in which the osmotic pressure of tomato juice is 1 to 2 atm., inhibition is sometimes complete; isotonic salt or sugar solutions have no effect whatever on germination (79). Kökemann (74) found the same for other fruit juices which inhibited even in 1: 10 dilution where the osmotic pressure was negligible.

(*c*) By yeast fermentation the sugar content of the juice (3.6% glucose) was reduced to nil (osmotic pressure less than 0.5 atm.). This juice still inhibited germination, but in an isotonic glucose solution treated similarly no inhibition was observed (Orig.).

(*d*) There is no correlation between the height of the leaf sap osmotic pressure and its inhibitory effect. Saps of different parts of *Typha* leaves show the same inhibition, but their osmotic value varies from 9.3 to 14 atm. (80).

(*e*) In some cases the inhibitor is volatile, as in *Vaccaria pyramidata* (14), *Cucumis sativus* (71), onion and garlic (Orig.), *Brassica nigra* (Orig.).

INHIBITION AND pH

Many authors (78–80, 135, 138, 139) raise the question as to whether the acidity of fruit juices is the cause of their inhibiting action. In a very extreme case, that of lemon juice, the extremely acid pH (about 2.5) makes it *a priori* probable that this factor may be responsible for the germination inhibition. Lemon juice with pH 2.4 inhibited the germination of wheat grains completely down to a dilution of 1:4; in a dilution of 1:8 the g.i. was 7%; in a dilution of 1:16, 32%; in a dilution of 1:32, 85%. After neutralization the respective germination figures were: dilution of 1:4, 1.5%, 1:8, 22%. This experiment shows that the pH alone was not enough to explain the inhibition, for then the influence of the neutralization would have been much more pronounced. The same is proved by germination experiments in buffer solution of different pH (78). In pH 2.27 the g.i. was 8.2%.

In experiments with artificial lemon juice (solution of 5.41% citric acid and 0.32% malic acid (pH 2.2)), complete inhibition was observed down to 1:4; and in 1:8, 6.3% germinated (131). These figures compare well with the germination percentage in natural juice. After neutralization complete inhibition was noted down to 1:4, and more or less normal germination in still lower dilutions. All this proves that pH contributes to inhibition but is not its only cause. Besides pH the osmotic pressure (23.44 atm. for natural, 34.5 atm. for neutralized undiluted juice) plays its part also, and the inhibition of the original concentration of undiluted natural juice can be explained by the combined effect of pH and osmotic pressure. But there is another factor involved whose presence is felt only where dilutions are used, since the inhibition caused by 1:8 diluted neutralized juice (4.2 atm.) cannot be explained by either pH or osmotic pressure alone.

A similar conclusion must be drawn for apple juice, when wheat grains germinated to 37% in the natural and to 45% in the neutralized juice as compared with 90% in the controls (138, 139), thus showing slight influence of the acidity but definitely ruling out the pH as the sole factor responsible for the inhibition.

For tomato juice and leaf saps the reasons why pH is of minor importance or no importance at all in causing inhibition are summed up as follows:

(*a*) Seeds germinated in buffer solution of a pH identical with that of the juices are less inhibited or not at all (66, 75).

(*b*) The inhibition is not affected or only slightly so by neutralization of the juice (79).

(*c*) There is no relation between the inhibitory action of leaf sap and its acidity. The sap with the lowest pH inhibited germination least. Saps with neutral or slightly basic pH are no less inhibitory than the acid ones (80).

CHEMICAL NATURE OF INHIBITORS

Hydrogen Cyanide $(H\text{–}C\equiv N \rightleftarrows H\text{–}N\equiv C)$

Amygdalin and other cyanophoric glucosides occur in many seeds, especially in those of the Prunaceae and Pomaceae (up to 2½%). It is hydrolyzed by emulsin to benzaldehyde, hydrogen cyanide and β-glucose. Leibach and Keil (85) were the first to point out that this process may be important in naturally occurring germination inhibitions.

Pure amygdalin under sterile conditions has a weak inhibitory effect (128). It has long been known, however, that HCN, set free from amygdalin, and the free CN group in general have a strong inhibitory effect on growth and germination (128): 0.0015% by volume suffices to inhibit the growth of *Pisum* seedlings (70), 0.1% to inhibit the germination of tomato seeds (72), 0.05% to check the growth of tomato seedlings (72), 0.024% to delay germination of *Lepidium* seeds (160). The germination of seeds is very much delayed (8, 72, 85) or completely inhibited as long as the seeds are kept in air containing HCN (narcotizing action according to 72, 85), but if removed from the HCN they retain their germinating capacity even after exposure of six days. In the presence of active charcoal the HCN set free by emulsin from the natural amygdalin is ineffective, for it is absorbed into the charcoal. The tissues of growing seedlings are much more sensitive to HCN than the seeds; the same concentration which only delays germination kills the root tissues of *Helianthus* seedlings (85).

It may therefore be assumed (72) that hydrogen cyanide is the germination inhibitor wherever seeds contain HCN or potential HCN-producing compounds. This has been proved for almond seeds. Two bitter almonds containing 1% to 3% amygdalin, placed in a petri dish together with 20 wheat grains, inhibited the germination of the latter completely, while two seeds of the sweet variety, containing only a trace of amygdalin had no effect and ten seeds depressed the g.i. only to 85% (148). The HCN in this case may be set free by the action of enzymes contained in the wheat grains, since it has been shown (85) that traces of HCN are freed from pure amygdalin by germinating test-seeds which apparently contain enzymes capable of hydrolizing amygdalin. The same effect as with bitter almond seeds is obtained with Aqua Amygdalorum amarum.

When bitter almonds are crushed their inhibitory effect is much stronger, and even only two crushed sweet almonds have a slight inhibitory effect because then the emulsin, which in the whole seeds is localized in separate cells, comes into direct contact with the amygdalin. This can be proved by the usual HCN-reaction which gives negative results with whole almonds but a strong reaction after one minute when crushed almonds are used (55). The same has been reported for *Prunus* seeds (85) where the whole seeds are ineffective, whereas the crushed embryos inhibit germination completely. When the seeds are washed for three days before being crushed, the inhibition disappears completely, an observation which in many cases may explain the fact that germination inhibitors are removed by washing or leaching the seeds for some time.

In *Crataegus* seeds HCN was liberated just before germination (39). This may indicate that the amygdalin, usually hydrolysed very slowly, was broken down very rapidly toward the end of the period of after-ripening and the HCN liberated from the tissue; then germination processes, once poisoned by HCN, were freed to function normally (140).

Ammonia

The water extract from sugar-beet seed balls inhibits or retards the germination of sugar beet and many other seeds (38, 45, 46, 135, 136) and is toxic to germinating seeds and seedlings (135). It has been stated (38) that this effect is due principally to the osmotic pressure of the extract caused by its inorganic constituents. But there are volatile substances present in the non-decolorized extracts which also cause inhibition (38). Stout and Tolman (135) found that ammonia, released by enzymes from the nitrogenous compounds of the extract, is involved. This is proved by the following facts:

(*a*) The chemical test shows that during germination ammonia is liberated from the seed-ball extracts (135, 136).

(*b*) The quantity liberated is 0.3–0.4 mg of free ammonia per cubic centimeter of extract. This is enough to account for the inhibiting and toxic effect of the extract, since in controls the same amount of ammonia, liberated from other sources, had exactly the same effect (135). Other authors also report the highly toxic effect of ammonia which is even more injurious to germination than HCN (8). This agrees with the records of some authors (103) that ammonia in a concentration of 1 : 24,000 hampers the germination of *Vicia Faba,* in 1 : 20,000 that of *Zea Mays.*

The hydrolytic agent responsible for the freeing of ammonia is present in sugar beet seed extracts and intact germinating seeds. Lettuce seed extracts also contain some hydrolyzing agent freeing ammonia. This offers an explanation for the germination inhibition observed when lettuce seeds are successively germinated on the same substratum (125).

Ethylene

Ethylene is liberated by many fruits, *e.g.,* apples and pears (33, 34, 106), as well as by whole plants. The amounts produced are fairly considerable (1.78 ml to 11.38 ml per kg of apples per 24 hrs (58)) and suffice to cause a very pronounced growth and germination inhibition.

Seeds of different species were put beneath jars of six to eight liter capacity together with two or three ripe apples. Germination of the seeds was completely inhibited or retarded (74, 75). The suggestion put forward (79) that the volatile inhibitor of tomato juice might be ethylene could not be proved. This volatile inhibitor is not formed when disinfectants are added and is developed only by the action of microorganisms (Orig.).

Mustard Oils

Observations and experiments with the strong inhibitors contained in different plants belonging to the Cruciferae support the suspicion that mustard oils are the inhibiting substances in such cases. This supposition was based on the following facts:

(*a*) The fruit of *Sinapis alba* is composed of two valves and a beak. The valves are dehiscent, and the beak bears one seed which remains enclosed within it. This seed does not germinate so long as it is within the beak. When taken out, it germinates immediately. Valves and beak have been shown to contain strong inhibitors (133, 165). The same holds true for *Sinapis arvensis* (148), *Brassica nigra* and other cruciferous fruits (133, 165). The inhibitor is volatile and can be partly isolated by steam distillation (148).

(*b*) Fifty whole seeds of *Brassica nigra,* placed in petri dishes, lowered the g.i. of 50 wheat grains in the same dishes to 23%. When the seeds were ground the g.i. of the wheat grains was only 6% (Orig.). The inhibitor set free from ground seeds is volatile.

(*c*) The juice of horseradish, radish and kohlrabi roots contains a strong volatile inhibitor (Orig.).

Now it is well known that mustard oils, mostly allyl-isothiocyanate ($CH_2{=}CH{-}CH_2{-}N{=}C{=}S$) and β-phenethyl-isothiocyanate ($C_6H_5{-}CH_2{-}CH_2{-}N{=}C{=}S$), are very common in all organs of plants belonging to the Cruciferae, especially in the genera *Brassica* and *Sinapis.* Seeds of *Brassica nigra* contain 0.7% of allyl-isothiocyanate which is extracted from them by steam distillation and sold as Oleum Sinapis (92% to 95% allyl-isothiocyanate). Germination experiments using this oil proved it to be a strong volatile inhibitor, inhibiting in the same concentration as the inhibitor of *Brassica* seeds (148) and adsorbed by charcoal in the same way. In the seeds the glucoside sinapin, not the free mustard oil, is hydrolyzed by myosin to d-glucose, potassium hyposulphat and allyl-isothiocyanate. This explains the fact mentioned under (*b*). β-phenethyl-isothiocyanate is very similar in effect to that of allyl-isothiocyanate. It is most interesting that seedlings of *Raphanus sativus, Brassica alba, B. oleracea* var. *capitata,* var. *gemmifera* and var. *caulocarpa* give off mustard oil vapors which kill potato test plants (34). All together, it is certain that natural allyl- and β-phenethly-isothiocyanate (and apparently other mustard oils belonging to the same chemical group) are germination inhibitors.

Organic Acids

Blastokolin is the germination-inhibiting substance postulated by Köckemann (74, 75) as being in the fruit flesh of apples, but Tetjurew (139) came to the conclusion that "blastokolin is nothing else than a mixture of malic and citric acid". These two acids are the most common in fruit juices and plant sap. The fruit juice of tomatoes contains 0.48% malic acid; of *Vitis vinifera,* 1.16%; of *Sambucus nigra,* 1.1%; of *Vaccinium Myrtillus,* 0.85% (147). The organic acid content is especially high in citrus fruit (5% to 6%).

While some inhibiting influence is exerted by these acids on germination through their control of pH, it is mainly by their intrinsic natures as specific acids that they are inhibitors. This is true also for other naturally occurring acids (43). Evidence for this lies in the fact that the pH of the lowest concentration causing complete inhibition is not significantly different from the pH of the highest concentration permitting normal germination. Such figures are as follows: citric acid, 1/100 (pH 2.39) and 1/2,000 (pH 2.93); malic acid, 1/200 (pH 2.57) and 1/1,000 (pH 2.87); salicylic acid, 1/1,200 (pH 2.45) and 1/6,000 (pH 3.30); acetic acid, 1/1,000 (pH 3.30) and 1/6,000 (pH 4.4); crotonic acid, 1/1,000 (pH 3.2) and 1/4,000 (pH 4.2). Since crotonic and acetic acids still inhibit completely at a pH of 3.2 or 3.3, whereas citric and malic acids do not inhibit any longer at a pH of 2.81 to 2.93, the pH cannot be held responsible for the inhibition caused by these two organic acids. There is, however, some influence of the pH in each case, for nearly always the inhibition is weaker when solutions of neutral salts of the different acids are used (43, 139).

The lowest concentrations for complete inhibition show that wherever the free acid content of inhibiting juices or sap reaches 1% for citric acid, 0.5% for malic acid, 0.2% for tartaric acid, 0.1% for acetic and crotonic acid, and 0.09% for salicylic acid, the inhibition is caused at least partly by these acids wholly apart from their influence on the pH.

Since different acids are always mixed in juices and sap, the synergistic effect of them must be taken into consideration too. Whereas a 1/2,000 dilution of citric or of malic acid alone causes no inhibition, the g.i. in a mixture of equal parts of these acids at this dilution is only 65% (43). The synergistic effect of a three-acid mixture is still more pronounced. A mixture of 0.025% citric, 0.025% tartaric and 0.01% salicylic acid (total acids 0.06%) exerts a much stronger effect than each acid alone in a concentration of 0.06% (43).

Besides the acids cited, cinnamic acid (trans-form) and some of its derivatives seem to be of special importance as inhibitors.

Trans-cinnamic acid formed by guayule plants is a growth- (12) and germination-inhibitor (130). Sodium cinnamate and dihydrocinnamic acid inhibit germination completely, even in concentrations of 0.01 mol. (124). Caffeic acid (3,4-hydroxy-cinnamic acid) and ferulic acid (3-methoxy-4-hydroxy-cinnamic acid) have been isolated from tomato juice (1) and are at least partly responsible for its germination-inhibiting action. Traumatic acid, the plant wound-hormone, is also a germination inhibitor (40).

Unsaturated Lactones

The fruit of *Sorbus aucuparia* contains a strongly inhibiting substance, parasorbic acid ($C_6H_8O_2$), which inhibits germination of *Lepidium* seeds in dilution of 1 : 1,000 (calculated on the pure substance) and gives at 1 : 10,000, 10% to 80% germination (82). It is present in the berries of the mountain ash in a concentration of at least 1 : 1,000 (Kjaer, according to 88), and belongs chemically to the unsaturated lactones.

Anemonin ($C_{10}H_8O_4$), another unsaturated lactone, found in various members of the Ranunculaceae, is a germination inhibitor, too (Kjaer, according to 88).

Coumarin ($C_6H_4OCOCH:CH$), another naturally occurring and widely distributed unsaturated lactone, is a strong inhibitor and was already mentioned by Sigmund (129, 130). In concentrations of 1 : 10,000 only 2% of the seeds germinate (82). On wheat coumarin is less effective, since the lowest concentration for complete inhibition is 1 : 8,000 (Orig.), proving that coumarin is one of the most potent inhibitors known.

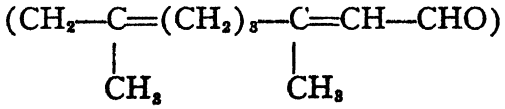
It is most interesting that coumarin brings about an induced dormancy in non-dormant lettuce seeds (109). After coumarin treatment the seeds react to dormancy-breaking agents, *e.g.*, thiourea, exactly as seeds do which are naturally dormant. It is possible, therefore, that coumarin is the natural germination inhibitor of lettuce seeds (125, 126). The inhibitor or inhibitors of tomato juice are, according to Veldstra (153), unsaturated lactones, too. He writes: "We isolated from tomato juice a liquid lactone and a crystalline acid, both of them inhibiting seed germination and according to their chemical properties belonging to the discussed series", *i.e.*, unsaturated lactones. No further evidence has yet been published.

Aldehydes

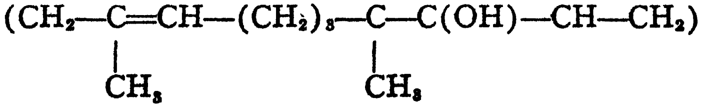
As far back as 1910 Mazé (101) reported that unripe grains of corn and unripe seeds of peas containing 50% to 60% of water do not germinate, but do so when dried artificially. This is not due to the relatively great quantity of water contained in the unripe grains, but to the presence of an inhibitor, for ripe seeds do not germinate in extracts of the unripe ones. This inhibition is attributed to acetaldehyde which is present in unripe (0.1 mg per 20 gr. of corn) and missing in ripe seeds. In germination experiments the same concentration of pure aldehyde inhibited germination. The same author (100) is of the opinion that seeds placed under water for some time lose their vitality because acetaldehyde accumulates.

Benzaldehyde (C_6H_5CHO), which is formed during hydrolysis of amygdalin, is a strong inhibitor (129, 130 and Orig.). The lowest concentration of complete inhibition is 1/2,000 to 1/3,000; of incipient inhibition, 1/8,000 to 1/10,000 (Orig.). Its vapours, too, are very inhibiting; 0.0006% by volume still inhibits germination of wheat grains completely. Benzaldehyde undoubtedly participates in the inhibition brought about by bitter almonds.

Salicylaldehyde ($O—HOC_6H_4CHO$) is an even slightly stronger inhibitor which finds its explanation in the fact that the OH group is also an active inhibiting group.

Citral

$$(CH_2—\underset{CH_3}{C}{=}(CH_2)_3—\underset{CH_3}{C}{=}CH—CHO)$$

present in lemon grass oil inhibits completely at 1/500 (130); its limit of complete inhibition is 1/1,000 to 1/2,000 (Orig.).

The related linalool

$$(CH_2—\underset{CH_3}{C}{=}CH—(CH_2)_3—\underset{CH_3}{C}—C(OH)—CH—CH_2)$$

has at 1/500 only a very slight inhibiting action (g.i. 62% (130)), showing that the CHO group is really responsible for the inhibition and appears to be active practically without any influence of the neighbouring groups.

Cianamalaldehyde (C_6H_5—CH═CH—CHO), too, is a very strong inhibitor (130), much more effective than cinnamic acid.

The same effect of the CHO-group is noteworthy in comparing the activity of anisicaldehyde (p-methoxybenzolaldehyde (p-$CH_3OC_6H_4CHO$) and anisic acid (p-methoxybenzoacid-p-$CH_3OC_6H_4COOH$) where in 1/500 dilutions the aldehyde depresses the g.i. to 8%, the acid to 39% (130). Other examples are: 1/200 mol crotonic acid, g.i. 98%; crotonaldehyde, 0%; salicylic acid 1/200 mol, g.i. 54%; salicylic aldehyde, 0% (Orig.).

Though from chemical considerations one should expect a strong inductive influence of the various constituents, it appears that as far as germination is concerned the specific nature of the isolated CHO group determines the extent of inhibition, while inductive and electromeric influences are of secondary importance.

Essential Oils

When the flavedo of a half or a whole lemon or orange peel is removed and put into a big petri dish in which a small petri dish is placed containing 50 wheat grains so that there is no direct contact between the wheat grains and the peel, the germination of the wheat grains is completely inhibited by the flavedo (Orig.). That the essential oil contained in the peel is the cause of this inhibition may be concluded for the following reasons:

(*a*) When the flavedo is peeled off and the oil glands are cut open by the peeling operation, the g.i. of 100 wheat grains is reduced to 18% by 20 cm^2 of peel used in the volatility experiment described above. If the peel is cut together with its albedo, and the glands remain uninjured as far as possible, the g.i. is 48%. When the peel is cut as in the first experiment but is washed thoroughly before being used, there is no inhibition at all (Orig.).

(*b*) When the essential oil of the peel is extracted by the usual steam distillation, the distillate inhibits strongly and the remaining peel loses its inhibiting influence (g.i. 90%, Orig.).

(*c*) The pure essential oil of lemon (Oleum Citri)—even when only traces are smeared on the inside of the cover of a petri dish—inhibits germination (148). All essential oils used (Oleum Coriandri, Rosmarini, Lavandulae, *etc.* (148)) show the same effect.

According to Sigmund (130), the descending order of inhibition is: Oil of cloves (2%), of peppermint (6%), of rosemary (6%), of rose geranium (6%), of caraway (7%), of thyme (18%), of fennel (21%), of lavender (26%), of rue (34%), of Eucalyptus (51%), of anise (32%). These percentages designate the g.i. on the third day.

Different fruits, seeds and leaves containing essential oils show the same inhibiting effect, even when not in contact with the test seeds. With the fruit of *Foeniculum vulgare* (148) and its essential oil (Oleum Foeniculi), exactly the same experiments as described for lemon peel can be made (148). One half gram of rosemary leaves used in the volatility experiment give a g.i. of 44% (Orig.).

The growth-inhibiting and killing effect of essential oils on plants has long been known (26, 61), and the plants producing the oils are much more resistant to their own oils than are other species. *Brassica* was killed by *Dictamnus* vapours after four days, whereas *Dictamnus* remained intact for 12 days when branches of the two plants were placed beneath the same bell jar (61). Vapours of essential oils are more effective in this respect than liquid oils or solutions. The same may be observed for germination inhibition (Orig.).

Since the groups of the so-called "essential oils" are heterogenous from a chemical point of view, we do not know which constituents are responsible for the inhibition. There are only some indications as to the active components (130):

(*a*) Aromatic and alicyclic hydrocarbons (styrene, p-cymene, d- and e-pinene, d- and e-limonene), alcohols (borneol, linalool, menthol, terpinhydrate) and acids are apparently not responsible for the inhibition, for all such substances did not inhibit germination at all or did so only very slightly. The only exception was terpineol which inhibited more strongly than the other compounds.

(*b*) Aldehydes (benzolaldehyde, salicylic aldehyde, citral, cinnamal-aldehyde, anisic-aldehyde), phenols (thymol, carvacrol, apiol, safrol) and ketones (carvone, camphor, fenchone, eucalyptol), which are present in many essential oils, perhaps in all, are strong inhibitors and may be responsible for the inhibition.

Aldehydes and phenols especially are to be considered the main active components, described as far back as 1893 as Ermüdungsstoffe (119), *i.e.,* metabolites which inhibit or retard growth, protoplasmic movement and other processes. Thymol, carvacrol, cinnamal-aldehyde and salicylic aldehyde are cited specially. Their "plasmatiring" action is explained as that of substitute poisons, a very modern idea indeed.

Alkaloids

Oppenheimer (110, 111) long ago assumed that the inhibition of tobacco seeds, when put near their seed capsules or leaves, might be caused by the alkaloids contained in the latter. All seeds and fruits known for their high alkaloid content are strong germination inhibitors (148), and it can be proved that the alkaloids are the main if not the only cause of inhibition in these cases. The reasons are:

(*a*) When the alkaloids are removed by suitable extraction methods (the alkaloid-containing extracts inhibit), the inhibition of the seeds from which the alkaloid was removed is greatly diminished (148).

(*b*) Pure alkaloids in concentrations much weaker than those in which they are contained in fruits and seeds, exert the same inhibition as the fruits and seeds themselves (148). (Coffee seeds contain 0.3% to 2.36% caffein; coca leaves, 0.25% to 0.6% cocain; seeds of *Strychnos nux vomica,* 1.12% to 2.23% strychnin and 2.3% to 3 9% total alkaloids; seeds of *Physostigma venenosum,* 0.17% physostygmin; cocoa seeds 1.05% to 2.34% theobromin and 0.05% to 0.36% caffein (147). (Compare the germination experiments made with 1/200 solutions of pure alkaloids!).

(*c*) In the genus *Trigonella,* only the alkaloid-containing species, *Foenum graecum,* inhibits germination. Only 20 seeds suffice. The seeds of the closely related species, *T. Kotschyi* and *T. monspeliaca,* which do not contain alkaloids, do not inhibit, even when 100 seeds are used (148).

Of all alkaloids investigated (43, 128, 148), only the following inhibit. (The percentage figures indicate the g.i. of wheat grains in 1/200 dilutions):

(*a*) Strong inhibitors: Cocain (0%), physostigmin (0%), caffein (2.5%), chinin (3.5%), cinchonin, cinchonidin, tropa acid, strychnin (25%), berberin and codein (35%).

(*b*) Weak inhibitors: Narkotin and scopolamin (58%), emetine (65%), papaverin (65%), ephedrin (73%), piperine and atropine (81%).

When more diluted, most alkaloids stimulate germination, as pointed out by many authors (see 37, where older literature is cited) who sometimes even write about the hormonal action of alkaloids.

CORRELATION BETWEEN INHIBITORY ACTIVITY AND CHEMICAL STRUCTURE OF THE INHIBITORS

Only the first steps have been taken to elucidate the relation between inhibitory effect and chemical constitution of inhibitors.

Mustard Oils

The group $-S-C\equiv N$ or $-N=C=S$ is four to five times more effective than the group $-C\equiv N$ or $-N=C$, since the limit of complete germination inhibition lies for allylcyanid and allylisocyanid around 1/1,500, for allylisothiocyanate and allylthiocyanate around 1/5,000–1/6,000 (Orig.). The same results are obtained with phenylisothiocyanate and phenylcyanid.

There is no difference between $-S-C\equiv N$ and $-N=C=S$ (Orig.).

The type of radical attached to the active group changes the inhibitory effect of the compound. The order of decreasing activity is: phenyl, allyl, ethyl (Orig.). The fungicidal effectiveness has shown another order of decreasing toxicity: allyl, phenyl, methyl, ethyl (157).

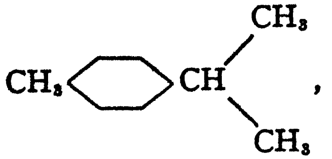
The introduction of halogen atoms enhances the inhibitory effect (Orig.).

Phenols and Organic Acids

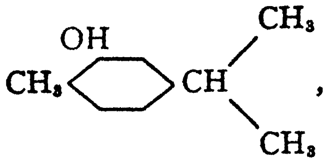
With phenols the inhibitory effect decreases generally with the increasing number of OH groups, *e.g.*, phenol, resorcinol, phloroglucinol. The results of Sigmund (130), obtained with *Vicia* and *Rapa* as test seeds, might indicate a position effect of the OH-group. The g.i. figures for *Vicia* are: catechol (orthodioxy-benzol) 2.1%, resorcinol (metadioxybenzol) 22%, hydroquinone (paradioxybenzol) 96%, pyrogallol (vicinal trioxybenzol) 21%, phloroglucinol (symmetric trioxybenzol) 93% (1/40 mol. solution, 5th day of germination).

In quite another way, too, it can be shown that the inhibition caused by phenolic compounds is a function of the active OH group and is largely independent of the nature of the structural nucleus to which it is attached.

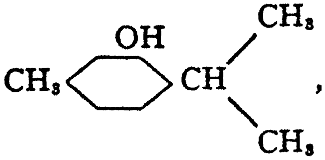
Cymol,



in 0.2% solution has a g.i. of 81%; carvacrol,



and thymol,



of 0% (130).

With aromatic carboxylic acids the inhibitory effect decreases with the increasing number of carboxylic groups.

The introduction of an OH group enhances the inhibition (1/200 mol. of benzoic acid 17%, salicylic acid 0%). If more OH groups are introduced the inhibition decreases (gallic acid 9.6%, protocatechuic acid 39%).

When there are side chains a double bond increases the activity (mentioned later for tropa and atropa acids). The same holds true for aliphatic carboxylic acids (129, 130, Orig.). Halogenation of the benzene ring increases the inhibiting action (benzoic acid 1/200 mol. germination inhibition of 21%, orthomonochlorbenzoic acid 0%).

Alkaloids

This group is much too complicated to permit ascertaining any relationship between structure and function. A few interesting facts should be mentioned, however. Atropin does not inhibit or does so only very weakly (128). From its cracking products atropa acid is a very strong inhibitor, tropa acid inhibits less, and tropin shows less inhibition than atropin (128). The increased activity of atropa acid in comparison to that of tropa acid is perhaps due to the presence of a double bond. The related compounds chinin, cinchonin and cinchonidin are all strong inhibitors.

PHYSIOLOGICAL ACTION OF THE INHIBITORS

Inhibition and Stimulation

All authors who have tried to elucidate the physiological action of the different juices and extracts containing inhibitors have found that inhibition is accompanied by a stimulation of germination and growth. At sufficiently high dilution, the inhibition of tomato juice is replaced by stimulation. This stimulation starts at a dilution of 1/8, reaches its peak at a dilution of 1/60 to 1/250 and disappears at 1/1,000 (79). Tobacco seeds germinated on tobacco seed capsules are at first strongly inhibited, but show a considerable stimulation after 20 days (111). The water extract of wheat grains stimulates after an initial inhibition lasting three or four days (107). Different seeds placed for some time into *Beta* seed ball extracts and then into water show a three- to four-fold increased germination and growth velocity (47). Fruits of *Helianthus, Avena* and *Triticum* excrete, together with an inhibitor, a non-specific substance stimulating germination (123).

There are three ways to explain the relation between inhibition and stimulation:

(*a*) The same substance inhibits in high and stimulates in low concentrations.

This is in agreement with the well known fact that hormones and poisons inhibit at high and stimulate at low concentrations. The action of different dilutions of tomato juice seems to confirm this supposition.

(*b*) Inhibition and stimulation are caused by two different substances.

The stimulating action of tomato juice is much more pronounced in dialysed than in undialysed juices, and the stronger the inhibition in undiluted juice, the less marked is the stimulation at high dilution (79). In wheat extracts the inhibiting action is eliminated by heating, but the stimulating factor remains (107). These facts point to the existence of two different substances which are present at the same time in the undiluted juices. The inhibitor is more sensitive to dilution than the stimulator whose action is concealed by the inhibitor in low dilutions and is manifest only at high dilution where the inhibitor is rendered ineffective.

(*c*) The inhibitor is transformed into a stimulator by a slight chemical change during germination.

In *Helianthus* the dry fruit in all its parts contains only inhibitors. When the different organs are isolated from seeds after imbibition of six hours, the plumule and radicle stimulate, the cotyledons and fruit coat inhibit initially, but after some time the inhibiting action of the cotyledons is reversed into stimulation. After an imbibition of 15 to 18 hours, all organs stimulate besides the fruit coat which shows an initial inhibition (123).

Ruge draws from these experiments the conclusion that during germination the inhibitors are transformed into stimulators as long as the different fruit parts are in organic contact. This transformation does not take place and the inhibitors remain unchanged when the different organs are extracted from dry fruits and imbibed separately. Ruge, together with other authors (150, 151), is of the opinion that auxins are not involved. They developed an interesting theory about the formation of inhibitors and transformation of them into stimulators. The following steps are supposedly involved:

(*a*) Hydrolysis of a nitril-glucoside of the amygdalin type and formation of HCN which is a known inhibitor.

(*b*) Addition of elementary sulphur to the HCN molecule by action of rhodanase and formation of HSCN which, according to Gemeinhard (55), is a stimulator.

We may mention here the interesting theory of Veldstra (152) concerning growth stimulators and inhibitors. He has shown how the same substance can inhibit or stimulate in different concentrations and produce changes in the structure of plasmic membranes. This may be analogous to our germination inhibitors and stimulators which—like the auxin in Veldstra's theory—could swell or condense the plasmic membrane, regulate permeability and hamper or stimulate different processes necessary for normal germination.

Germination and Growth Inhibition

Germination- and growth-inhibition are nearly always associated with one another. When seeds in different stages of germination are transferred from water to tomato juice, their growth is inhibited. In a very advanced stage they continue to sprout, but the radicles turn black and degenerate (79). Generally the roots are much more sensitive to the inhibition than the coleoptile, plumule or young sprouts. The same holds true for wheat grain extract and its inhibitor (107). Froeschel (47) states, concerning the *Beta* seed inhibitor, that germination inhibition is only one aspect of the inhibition problem and that the second aspect is the considerable inhibition and retardation of growth. Barley extract inhibits germination and growth (83). *Beta* seed-ball extract inhibits considerably all cell elongation in the *Avena* coleoptile cylinder test (38). In the *Avena* curvature test, tomato juice, parasorbic acid and anemonin retard growth (88). Different alkaloids (caffein < chinin < strychnin < atropin < ephedrin < codein < nicotin) have the same effect (43). Growth inhibition is still apparent in dilutions in which there is no longer any trace of germination inhibition. Certain leaf saps must be diluted to one-tenth of their normal strength to eliminate germination inhibition, but to 1/25 or 1/50 to eliminate growth inhibition. The respective figures for coumarin are 1/40,000 and 1/100,000 (158 and Orig.); for phenylisothiocyanate, 1/50,000 and 1/80,000 (Orig.).

All these facts may indicate that germination inhibition is only an inhibition of the first phases of growth.

There are some observations which do not fit into this picture, namely, that some growth inhibitors are inactive upon germination (96), and that sometimes the same substance acts differently on growth and germination (96). Today the experimental data are too scant to decide this question. "The identity of the inhibition of growth and germination has never been proved, although it has tacitly been assumed by several authors" (38).

Inhibitors and Auxins

Many authors (31, 84, 105, 108, 120) have found auxins in seeds, and some (84, 105) have shown that the auxin content of seeds decreases during germination.

If the auxin present in seeds were to function as a "germination hormone", the action of inhibitors could be explained by auxin destruction or inactivation. But, can auxins be considered as germination hormones? Veh and Söding (151) answer this question in the negative. The germination inhibition caused by apple seed endosperm can be explained neither by inactivation of its auxin, since the endosperm contains active auxin, nor by any inhibiting influence of the auxin, for the quantities contained therein are too small for that. Furthermore nearly all authors have found that different growth hormones inhibit germination only at higher concentrations than are found naturally in plants and are of no effect in lower concentration (review in 6, 7). Only when seeds were used which normally exhibit some delay in germination or old seeds whose germination capacity was decreased, was there some stimulation of the treated seeds (2, 6, 7, 86).

There are a number of facts supporting the second supposition that inhibitors destroy or inactivate growth hormones or lower the response of cells to them. Beet-seed extract, for instance, applied together with heteroauxin in the *Avena* coleoptile cylinder test decreased the activity of the latter (38).

Mixtures of growth hormones, on one hand, and tomato juice, parasorbic acid and anemonin, on the other, when applied in the *Avena* curvature test, suppress or decrease the curvature of the coleoptile (88). This explains the shape of the curves produced by plant extracts which apparently contain a growth-retarding substance together with a growth-promoting one (3-indol-acetaldehyde). In tomato juice Larsen (87) observed three different growth inhibitors and assumed that one of them is identical with the acid germination inhibitor present in this juice, a supposition supported by Juel (69). He found that the acid extract of tomato juice inhibits growth of coleoptiles and roots. This inhibitor does not destroy the auxins but counterbalances their activity. Since Konis (80) found germination inhibitors in nearly all leaf sap, and other authors (53, 92, 134, 154, 155) have noted growth inhibitors in the extracts of different parts of numerous plants which act as antagonists to growth-promoters, there is a case for the identity of germination and growth inhibitors.

Caffein applied together with heteroauxin in the *Avena* test affects the activity of the latter very strongly (43). It is noteworthy that the growth inhibition is stronger when caffein and heteroauxin are applied together or one after the other than when caffein is used alone (43).

An interesting theory concerning HCN as inhibitor and heteroauxin as stimulator is proposed by Keil (72) for apple seeds. According to him, apple seeds do not germinate normally because HCN in the surroundings of the embryo narcotises all the cells and lowers their response to heteroauxin which is not attacked by HCN. When the HCN is removed, the seeds germinate, for then the "narcosis" of the cells to heteroauxin is abolished.

Effect of Inhibitors on Germination Capacity

When wheat grains are placed in tomato juice for varying lengths of time and then transferred to water, germination is retarded, even after only two hours in the juice. Submersion of 50 hours or more renders the grains incapable of germination (79). The same holds true of the *Beta*-inhibitor where seeds of *Antirrhinum* and *Medicago* are irretrievably damaged after seven days stay in the extract. The seeds of 11 other species germinated properly, even after a long stay in the extracts, when the inhibitor was removed by washing the seeds before their transfer to water.

Inhibition and Light

A very peculiar feature of inhibitors worthy of further elucidation is their reaction to light, even when the test seeds germinate in light and darkness alike. The inhibition of wheat grains caused by wheat extracts is stronger in light than in darkness (107). *Lepidium* and *Gypsophila* seeds are much more inhibited by *Beta* extracts in the presence of light. Darkness diminishes inhibition considerably (47). Decolorised *Beta* extracts, which apparently contain only inorganic salts as inhibitors, show the same light effect (38). Even with isotonic salt solutions the inhibition is much more pronounced in light (38). A similar effect is observed with seeds whose germination is normally influenced by light. Seed extracts of *Phacelia tanacetifolia,* a darkness-germinator, inhibit only in weak light and are ineffective in darkness (97, 114). Dormant lettuce seeds are 20° to 25° C-darkness germinators and do not germinate in light. Non-dormant seeds are made light-sensitive by treatment with coumarin, *i.e.,* when treated with coumarin in light or when light strikes the seeds after coumarin treatment, while the seeds are still wet, germination is inhibited.

There can be no doubt that the problem of seeds whose germination is influenced by light or darkness is closely related to the problem of inhibitors. There are many indications (*e.g.,* 54, 141) that in light-germinators, *e.g., Chloris ciliata,* an inhibitor is formed during germination which is destroyed by light of a certain wavelength or by application of certain chemicals. The germination-stimulating influence of the light could be explained as "an inhibition of an inhibitor" (54).

With darkness germinators, *e.g., Phacelia tanacetifolia,* the inhibitor could be a photodynamic substance which inhibits only in the presence of light (97). We may mention in this connection that Metzner (104) found photodynamic fluorescent substances in many different seeds. It would be most valuable to follow this line of research, connecting the inhibitors with the highly interesting problem of radiation influence on germination.

There are two other very interesting angles to the problem of radiation influence on inhibitors. The growth hormone auxin-a-lactone is transformed to lumi-auxin-a-lactone under the influence of light. Lumi-auxin is supposed to be an unsaturated lactone, acting, like most unsaturated lactones, as a growth inhibitor (153). This is in harmony with the above cited seeds, where inhibitors are activated by light, and with coumarin- (an unsaturated lactone!) treated lettuce seeds, where germination is inhibited by light. Secondly, trans-cinnamic acid, formed by guayule plants (11, 12), is a strong growth-inhibitor (1 mg per liter is active), while cis-cinnamic acid is a growth hormone, and trans-cinnamic acid is transformed into cis-cinnamic acid by ultra-violet radiation (163). These facts offer a parallel to those seeds where light produces an "inhibition of an inhibitor".

Inhibition, Swelling, Respiration, Enzyme Activity

Inhibitors could function by hampering the water intake of seeds. All experiments in this direction (79, Orig.) proved that water intake is not influenced at all or is only very slightly depressed by inhibitors and is decidedly of no importance in explaining the action of germination inhibitors. There is in most cases an influence of the inhibitors on respiration. Respiration decreases about 10% in seeds treated with tomato juice (79). Barley seeds treated with their own inhibitor show much decreased respiration (83), but the respiration of *Beta* seeds soaked in the decolorised extract is not affected during the first four hours.

As far as hydrocyanic acid is concerned, the inhibition caused by it is certainly related to the well known strong inhibiting effect of this substance on respiration.

Konis (79) reports that the addition of juice to diastase *in vitro* does not alter its activity, whereas other authors (83) report decreased diastase activity of barley grains treated with their own inhibitor.

Inhibition, Oxygen, Carbon Dioxide

The role of oxygen and carbon dioxide in inhibition was recently discussed by Thornton (see 140 for literature). Secondary dormancy, *i.e.*, delayed and inhibited germination, can be induced in many seeds by lowering the normal oxygen supply. This happens when the oxygen in the atmosphere surrounding the seeds is replaced by carbon dioxide or when the seed coats are impermeable to gases. Then the oxydizing system is greatly inhibited and intermediate products of partial anaerobic respiration accumulate, *e.g.*, acetaldehyde (101) and many other toxic substances. These bring about dormancy, *i.e.*, they inhibit germination (140 and literature cited therein). These presumably accumulated inhibiting substances tie up the question of induced dormancy with our problem of germination inhibitors.

Thornton tries to explain in the same way the known effects of high and low temperature on germination and the interruption of dormancy: "High temperature storage increases the dormancy as the hydrolyzing system remains more active than the oxidizing one. Low temperatures retard the accumulation of inhibitors and lead to germination".

This theory should be used as a working hypothesis in further experiments, for it associates inhibitors, respiration, oxygen, CO_2 and different temperatures with germination and dormancy. It is noteworthy in this respect that recent observations have recorded (66) that 2,4-D, one of the most potent artificial inhibitors, affects aerobic respiration. Barley seeds germinating in an atmosphere of oxygen after treatment with 2,4-D, cannot use the O_2 and respire in an anaerobic way, as do untreated seeds germinating under low O_2-pressure.

Inhibition and Permeability

Essential oils decrease the protoplasmic permeability of *Elodea* and *Spirogyra* (137), but after some time the permeability is increased, so that water drops are excreted (Gigliogli, cited in 137). Later the cells die. It is possible that the inhibitory activity of essential oils is connected with their effect on permeability. Inasmuch as experimental data are scant, it would be desirable to pursue this line of research, especially since Veldstra (152) has put forward his interesting theory.

BIOLOGICAL FUNCTION OF INHIBITORS

The main biological functions of germination inhibitors are prevention, by self-inhibition, of premature germination; extension of the germination period over a long time; and suppression of germination of other species in the surrounding of the plants producing inhibitors.

Prevention of Premature Germination

If seeds were to germinate inside the fruits that contain them, one of their main biological functions—dispersal of the species over a wide area—could not be fulfilled. Such premature germination is prevented by the inhibitors within the seeds themselves. Another function would be the prevention of germination of seeds not yet ripe, if Mazé's experiments (101) can be affirmed.

Extension of the Germination Period Over a Long Time

In cases such as *Sinapis,* some of the seeds remain enclosed in the fruits and do not germinate, while other seeds fall out and germinate immediately. The enclosed seeds germinate only after the inhibitor contained in the fruit coats has been washed out by rain or the fruit coat has been destroyed by the action of microorganisms. This means that the germination of the seeds produced in one year is protracted over at least two years. If conditions are not favourable for such species during one year, germination the second year is assured by the reserve of germinable seeds remaining for the following year. The phenomenon of weeds reappearing in a field after a lapse of time may be explained in such a way.

Suppression of Germination and Growth of Other Species

Fröschel and Funke (48, 49) and Ullman (148) have shown that seeds containing inhibitors inhibit germination of other seeds when placed with them in pots containing soil or when planted directly in soil. *Agrostemma Githago* seeds do not germinate and the seedlings do not develop in soil treated with *Beta* seed extract. Wheat grains do not germinate when planted with *Viola* seeds. *Melandrium* and rye grass do not develop when *Beta* seeds are present in the same pot. Most seeds containing essential oils, alkaloids or glucosides inhibit germination and development of other seeds which may be in their vicinity. Wheat and rye grains suppress the germination of weed seeds, such as *Anthemis arvensis* and *Matricaria inodora,* when planted in the same pot (116). The phytosociological importance of this phenomenon has been pointed out by some authors (48, 49, 148).

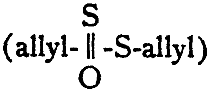
Germination and growth inhibitors are not restricted to seeds and fruits, but are secreted also by leaves and roots. Taking as a starting point the fact that parks of *Robinia pseudoacacia* are nearly void of all other vegetation, Waks (156) has shown that the bark and wood of *Robinia* contain substances hindering the growth of barley. Leaves of *Artemisia Absinthium* excrete ethereal oils and the alkaloid absinthin which inhibits the development of seedlings of *Foeniculum vulgare* and other species within a distance of one meter (10, 51). In one case (*Levisticum officinale*) the seedlings were even killed by the secretions of the absinthe plants themselves (51). Fresh leaves of *Artemisia Absinthium,* when dug into the soil, reduced the percentage of germination of a number of species (51).

Since the number of reports of plants excreting substances inhibiting germination and toxic to growth (11, 12, 106) is increasing, this phenomenon may well be of considerable biological and phytosociological importance.

INHIBITION, ANTIBIOTICS, MUTAGENOUS SUBSTANCES, PHYTONCIDES

Some inhibiting juices and most of the chemical groups to which inhibitors belong are known also as "antibiotics", "mutagenes", "phytoncides" or "insecticides", or are used in medicine because of their special physiological activities. Tomato juice, for example, kills *Paramaecium caudatum* (113). Space permits us to consider in this respect only mustard oils, unsaturated lactones, essential oils and alkaloids.

Mustard Oils

As far back as 1900 Beijerinck (9) found that "Benzylsenfoel" inhibits the growth of *Saccharomyces.* Seeds of *Brassica oleracea* contain an antibiotic principle belonging to the mustard oils (94), and the resistance of crucifers to clubroot disease (*Plasmodiophora brassicae*) is attributed to their content of such oils (122), a supposition supported by experiments showing the fungicidal activity of allylisothiocyanate and β-phenethylisothiocyanate (64, 65). The mustard oils and their vapours are highly toxic to different fungi in concentrations as low as 10 p.p.m. (115, 157, *etc.*). Vapours from crushed garlic show bacteriocidal effects (98, 159) which are attributed to allicin  $(\text{allyl-}\overset{S}{\underset{O}{\|}}\text{-S-allyl})$ (19, 20, 21). Allylthiocyanate is a "mutagene" with *Drosophila,* producing mutations (3). Treatment with allylisothiocyanate vapours causes structural changes in somatic chromosomes of *Drosophila* (132).

Kolle and co-workers (77), long before the Russian authors invented their "phytoncides", described the strong inhibiting action of vapours of different mustard oils in garlic, onion, radish and horseradish on animal tissue cultures and on bacteria. Microglia cells, for instance, are killed after an exposure of only two minutes to vapours of allylmustard oil given off by a 1 : 1,000 solution (77). Vapours are inhibitory at much greater dilution than are solutions (77), as is evidenced by the effects of essential oils (61 and Orig.).

Volatile "phytoncides" found in bulbs of onion and garlic have bacteriocidal and protistocidal properties, are lethal to yeast and eggs of mollusks and frogs, and have been used in medicine for keeping wounds clean and for stimulating regeneration of tissues (142, 143, 146, *etc.*). It seems certain that the phytoncides from garlic and onion are mustard oils. Garlic and onion have been used since time immemorial in folk medicine as disinfectants in stomach and intestinal infection.

Unsaturated Lactones

The antibiotics patulin, expansin, penicillic acid, clavonin, *etc.*, and perhaps penicillin itself, contain an unsaturated lactone ring (22, 82, 153, 158). Anemonin and protoanemonin are antibacterial agents (5). Cardiac glucosides, *e.g.*, digoxigenin, gitoxigenin and the very potent strophantidin, have an unsaturated lactone ring. Besides their physiological activity on the heart action they are antibiotic, delaying the souring of milk, and show sterilising properties (153).

Bufotalin and bufotoxin, the most important toad poisons, and many insecticides (153) are unsaturated lactones.

Parasorbic acid and related unsaturated lactones inhibit growth of bacteria, epithelial tissue and fibroblasts (82). This action is attributed to their interference with cellular proliferation because of their reactivity with SH groups essential to enzyme function (59). This mechanism could explain their germination-inhibiting action as well.

Medawar and co-workers (102) isolated from malt extracts a differential growth inhibitor, apparently the unsaturated lactone of δ = oxy = $\alpha\beta$ hexenoic acid.

Many coumarin derivatives act as anesthetics (153), and perhaps the anti-coagulant action of di-coumarol, which is also an antibiotic (57), is based on its being an unsaturated lactone.

Essential Oils

Essential oils have long been known and used as disinfectants because of their antibiotic action, especially against bacteria (18, 23). Focke (44) stated as far back as 1881 that plants producing essential oils are not attacked by parasitic fungi. Glands containing essential oils are protected against putrefaction. Leaves of *Rosmarinus, Salvia officinalis* and *Mentha viridis* are completely destroyed after being placed in pond water for four months, but the glands are left intact (36). Essential oils are very toxic to man and other animals. With this fact as a basis, Detto (36) believes that the biological function of essential oils lies in the protection they give plants against being eaten by animals. Such oils are "phytoncides", too, for different Russian authors (142, 143, 146) have found a strong bacteriocidal and protistocidal action in those of orange, lemon, mandarine leaves, *etc.* In their opinion "phytoncides" are not identical with essential oils but are components or derivatives of them.

Alkaloids

Drugs containing alkaloids are the oldest poisons, remedies and stimulants known to man, used even in prehistoric times (147). All of them are known for their outstanding physiological activity which is based partly on their antibiotic properties (chinin), partly on the inhibition and poisoning of important physiological processes. Only one example, colchicine, need be cited, the modern means of inducing artificial polyploidy. It arrests spindle formation in animal and plant cells and inhibits separation of sister chromosomes in plant cells, slowing down metabolic processes at the same time (35).

Since many germination-inhibiting substances are, as indicated, effective as inhibitors of all kinds of physiological processes in plants and animals, it is probable that they all affect one or more very basic reactions common to all living organisms. The mechanism affected may be of the kind assumed by Veldstra, *i.e.*, condensation or dilation of the plasmic membrane, which would change the permeability, or it may be an attack on some steps of the respirational system, or it may be a substitution reaction of the type supposed by Cavallito and Haskell (22). One thing is sure—in tackling the problem of germination inhibitors and of inhibitors in general, one touches a basic biological phenomenon. Further research into this really fascinating problem, which was already known to Albertus Magnus (76), is certain to yield most interesting results.

SUMMARY

The presence of germination-inhibiting substances in plants seems to be a wide-spread phenomenon. They occur in all parts of plants —in fruit pulp, fruit coats, endosperm, seed coat, embryo, leaves, bulbs and roots. They are non-specific in their effects.

Besides inhibitors, high osmotic pressure and acid pH are often partly responsible for the germination inhibition caused by sap, juices and extracts. The main known inhibitors are the following substances or belong to the following chemical groups: hydrogen cyanide, ammonia, ethylene, mustard oils, organic acids, unsaturated lactones, aldehydes, essential oils and alkaloids.

In some of these groups a relation between inhibiting activity and chemical structure of the inhibitor can be established.

Germination inhibition is nearly always accompanied by stimulation of germination. Sometimes inhibition and stimulation appear in different concentrations, sometimes one after the other in the same concentration.

Germination inhibitors seem to fulfill a biological function and must be taken into consideration as important factors in phytosociology.

There seems to be a relation between germination- and growth-inhibitors, antibiotics, mutagenes and phytoncides which may be based in common on some basic physiological process or processes.

ACKNOWLEDGMENT

My thanks are due to Dr. James Bonner, California Institute of Technology, who encouraged me to write this paper, and to my cooperators and friends, Dr. Y. Leibowitz, Dr. A. Katschalsky, Dr. E. Konis and R. Richter, for help in preparing the manuscript.

Thanks are due also to Dr. Fulling who undertook the difficult task of improving the English in my manuscript.

REFERENCES

1. Akkerman, A. M. and Veldstra, H. The chemical nature of Köckemann's blastocholine from *Lycopersicon esculentum* Mill. Rec. Trav. Chim. 66: 411–412. 1947.
2. Amlong, H. U. and Naundorf, G. Ueber einige praktische Anwendungen der pflanzlichen Streckungstoffe. Forschungsdienst 4: 417–428. 1937.
3. Auerbach, C. and Robson, I. M. Production of mutations by allyl thiocyanate. Nature 154: 81. 1944.
4. Axentjev, B. N. Ueber die Wirkung von Samenauszügen auf das Keimen von Samen. Jour. Soc. Bot. Russ. 22: 291–306. 1927.
5. Baer, H., Molden, M. and Seegal, B. C. The nature of the antibacterial agent from *Anemone pulsatilla.* Jour. Biol. Chem. 162: 65. 1946.
6. Barton, L. V. Some effects of treatment of non dormant seeds with certain growth substances. Contr. Boyce Thompson Inst. 11: 181–205. 1939/41.
7. ———. Some effects of treatment of seeds with growth substances on dormancy. Contr. Boyce Thompson Inst. 11: 229–240. 1939/41.
8. ———. Toxicity of ammonia, chlorine, hydrogen cyanide, hydrogen sulphide and sulphur dioxide gases. IV. Seeds. Contr. Boyce Thompson Inst. 11: 351–363. 1939/41.
9. Beijerinck, M. W. Ueber die Wirkung des Benzylsenfoels auf das Wachstum des Kahmpilze. Centralbl. Bakteriol. II. Abt. 6: 72. 1900.
10. Bode, H. R. Ueber die Blattauscheidung des Wermuts und ihre Wirkung auf andere Pflanzen. Planta 30: 566. 1939.
11. Bonner, J. Further investigation of toxic substances which arise from guayule plants: relation of toxic substances to the growth of guayule in soil. Bot. Gaz. 107: 343–351. 1946.
12. ——— and Galston, A. W. Toxic substances from the culture media of guayule which may inhibit growth. Bot. Gaz. 106: 185–198. 1944.
13. Borris, H. Die Zusammenwirkung von Substrat, Temperatur und Licht bei der Keimung von *Vaccaria pyramidata.* Ber. Deut. Bot. Ges. 54: (11)–(13). 1936.
14. ———. Ueber das Wesen der Keimungsfoerdernden Wirkung der Erde. Ber. Deut. Bot. Ges. 54: 472–486. 1936.
15. ———. Ueber die inneren Vorgänge bei der Samenkeimung. Jahrb. Wiss. Bot. 89: 254–339. 1941.

16. Borthwick, H. A. and Robbins, W. W. Lettuce seed and its germination. Hilgardia 3: 275–304. 1928.
17. Brodersen, R. and A. Kjaer. The antibacterial action and toxicity of some unsaturated lactones. Acta Pharmacol. et Toxicol. (Copenhagen) 2: 109–120. 1946.
18. Cadéac et Mennier. Action antiseptique des essences. Ann. Inst. Pasteur 220: 1889.
19. Cavallito, C. J. and Bailey, J. H. Allicin, the antibacterial principle of *Allium sativum*. I. Isolation, physical properties and antibacterial action. Jour. Am. Chem. Soc. 66: 1950–1951. 1944
20. ————, Buck, J. S. and Suter, C. M. Allicin, the antibacterial principle of *Allium sativum*. II. Determination of the chemical structure. Jour. Am. Chem. Soc. 66: 1952–1954. 1944.
21. ————, Bailey, J. H. and Buck, J. S. The antibacterial principle of *Allium sativum*. III. Its precursor and "essential oil" of garlic. Jour. Am. Chem. Soc. 67: 1032–1033. 1945.
22. ———— and Haskell, T. H. The mechanism of antibiotics. The reaction of unsaturated lactones with cysteine and related compounds. Jour. Am. Chem. Soc. 67: 1991–1994. 1945.
23. Chamberland. Les essences au point de vue de leurs propriétés antiseptiques. Ann. Inst. Pasteur 153: 1887.
24. Copisarev, M A new method of fruit and vegetable preservation. The metabolism of apples. Ind. & Chem. Eng. 54: 283. 1935.
25. Cornevin, Ch. Action de poisons sur la germination des graines des végétaux dont ils proviennent. Comp. Rend. Acad. Sci., Paris 113: 274–276. 1891.
26. Coupin, H. La toxicité comparée des essences végétales. Comp. Rend. Acad. Sci., Paris 152: 529–531. 1911.
27. Cox, L. G, Munger, H. M. and Smith, E. A. A germination inhibitor in the seed coats of certain varieties of cabbage. Am. Jour. Bot. 31: 93. 1944.
28. ————, ————, and ————. A germination inhibitor in the seed coats of certain varieties of cabbage. Pl. Phys. 20: 289–294. 1945.
29. Crocker, W. Mechanism of dormancy in seeds. Am. Jour. Bot. 3: 99–120. 1916.
30. Czapek, F. Antifermente im Pflanzenorganismus. Ber Deut. Bot. Ges. 21: 229–242. 1903.
31. Dagys, J. Die Hefenwuchsstoffe in Maiskeimlingen. Protoplasma 28: 205. 1937.
32. Davies, W., Atkins, G. A. and Hudson, P. C. B. The effect of ascorbic acid and certain indole derivates on the regeneration and germination of plants. Ann. Bot. 1: 329–351. 1937.
33. Denny, F. E. Testing plant tissue for emenations causing leaf epinasty. Contr. Boyce Thomps. Inst. 7: 341–347. 1935.
34. ————. Leaf epinasty test with volatile products from seedlings. Contr. Boyce Thomps. Inst. 9: 431–438. 1937–38.
35. Dermen, H. Colchicine polyploidy and technique. Bot. Rev. 6: 599–635. 1940.
36. Detto, C. Ueber die Bedeutung der Aetherischen Oele bei Xerophyten. Flora 92: 167–224. 1903.
37. Dragone Testi, G. Azione degli alcaloidi sulla germinazione dei semi. Ann. di Bot. (Torino-Roma) 19: 1–8. 1931.
38. Duym, C. P. A., Komen, J. G., Ultée, A. J. and Von der Weide, B. M. The inhibition of germination caused by extracts of seed balls of the sugar beet (*Beta vulgaris*). Proc. Kon. Ned. Akad. Wet. 50: 527–535. 1947.

39. Eckerson, S. A physiological and chemical study of after ripening. Bot. Gaz. **55**: 286–299. 1913.
40. English, J., Bonner, J. and Haagen-Smit, A. J. The wound hormones of plants. IV. Structure and synthesis of traumatin. Jour. Am. Chem. Soc. **61**: 3434–3436. 1939.
41. Evenari, M. On germination inhibitors. I. Introduction. Pal. Jour. Bot. Jerus., Ser. 2: 1–5. 1940.
42. ————, Konis, E. and Ullmann, S. B. The inhibition of germination. Chron. Bot. **7**: 149–150. 1942–43.
43. ————, Ullmann, S. B. and Teltscher, L. On germination inhibitors. VII. Organic acids and alcoloids as inhibitors. Pal. Jour. Bot. Jerus., Ser. [In press].
44. Focke, W. O. Schutzmittel der Pflanze gegen niedere Pilze. Kosmos **10**: 414. 1881/82.
45. Froeschel, P. Onderzoekingen over de physiologie van de Kieming. I. Remstoffen. Naturwet. Tijdschr. **21**: 93–116. 1939.
46. ————. Remstoffen van zaden en hun invloed op het Kiemingspercentage. Med. Landbouwh. Gent **7**: 238–253. 1939.
47. ————. Untersuchungen zur Physiologie der Keimung. II. Hemmstoffe. Biol. Jaarboek Gent **7**: 73–116. 1940.
48. ———— and Funke, G. L. Een poging tot experimenteele plantensociologie. I. Naturwet. Tijdschr. **21**: 348–355. 1939.
49. ———— and ————. Ein Versuch zur experimentellen Pflanzensoziologie. II. Biol. Jaarbock Gent **7**: 267–273. 1941.
50. Fukaki, S. Ueber die Frage der Beeinflussung des eigenen Furchtsaftes auf die Samenkeimung. Bull. Sci. Fac. Terkult. Kjusu Imp. Univ. **4**: 1930.
51. Funke, G. L. The influence of *Artemisia Absinthium* on neighbouring plants. Blumea **5**: 281–293. 1943.
52. Funke, H. Beiträge zur Kenntniss von Keimung und Bau der Mistel. Beih. Bot. Centralbl. **59A**: 235–274. 1939.
53. ————. Ueber Hemm- und Wuchsstoffe des keimenden Maiskorns. Jahrb. Wiss. Bot. **91**: 54–82. 1943.
54. Gassner, G. Beiträge zur Frage der Lichtkeimung. Zeits. Bot. **7**: 609–661. 1915.
55. Gemeinhardt, K. Beiträge zur Kenntniss des Rhodangehalts der Pflanzen. Ber. Deut. Bot. Ges. **56**: 275–297. 1938.
56. Gombert, J. Keimungshemmende Stoffe in Gerste. Wochens. Brauerei **58**: 211–213. 1941. [Not seen].
57. Goth, A. The antibacterial properties of dicumarol. Science **101**: 383. 1945.
58. Hansen, E. Quantitative study of ethylene production in apple varieties. Pl. Phys. **20**: 631–635. 1945.
59. Hauschka, Th., Toennies, G. and Swain, A. P. The mechanism of growth inhibition by hexenolactone. Science **101**: 383–385. 1945.
60. Heinricher, E. Samenreife und Samenruhe der Mistel (*Viscum album*) und die Umstände, welche die Keimung beeinflussen. Sitzber. Akad. Wiss. Wien, Math.-Nat. Kl. 1 Abt. **121**: 573. 1912.
61. Heller, A. Ueber die Wirkung aetherischer Oele und verwandter Körper auf die Pflanze. Flora **93**: 1–35. 1904.
62. Hemberg, T. Wachstumshemmende und Wachstumsfördernde Stoffe bei der Kartoffel. Ark. Bot. **331B**: 1–3. 1946.
63. ————. Studies of auxin and growth inhibiting substances in the potato tuber and their significance with regard to its rest period. Acta Horti Berg. **14**: 133–220. 1947.
64. Hooker, W. J., Walker. I. C. and Link, K. P. Effects of two mustard oils on *Plasmodiophora brassicae* and their relation to resistance to clubroot. Jour. Agr. Res. **70**: 63–78. 1945.

65. ————, ———— and Smith, F. G. Toxicity of beta-phenethylisothiocyanate to certain fungi. Am. Jour. Bot. **30**: 632–637. 1943.

66. Hsueh, Y. L. and Low, C. H. Effects of 2,4 D on seed germination and respiration. Science **105**: 283–285. 1947.

67. Ivanovics, G. and Horvath, S. Raphanin, an antibacterial principle of the radish (*Raphanus sativus*). Nature **160**: 297–298. 1947.

68. Jäger, G. Ueber Ermüdungsstoffe der Pflanzen. Ber. Deut. Bot. Ges. **13**: 70–72. 1895.

69. Juel, I. Studies on a growth retarding substance in tomato. Dansk Bot. Arkiv **12**: 1–16. 1946.

70. Jungmann, W. Physiologisch-anatomische Untersuchungen über die Einwirkung von Blausäure auf Pflanzen. Ber. Deut. Bot. Ges. **39**: 84–87. 1921.

71. Kaufmann, E. Beiträge zur Keimungsphysiologie von *Cucumis sativus* in Zusammenhang mit dem Wuchsstoffproblem. Planta **33**: 516. 1943.

72. Keil, I. Die Bedeutung der Blausäure bei der Samenkeimung. Jahrb. Wiss. Bot. **88**: 345–372. 1939.

73. Kisser, J. and Possnig, J. Unterschungen über den Einfluss gehemmter und geförderter Sauerstoffaufnahme auf Samenkeimung und Keimlingswachstum. Beitr. Biol. Pfl. **20**: 77–104. 1932.

74. Koeckemann, A. Ueber eine Keimungshemmende Substanz in fleischigen Früchten. Ber. Deut. Bot. Ges. **52**: 523–526. 1934.

75. ————. Zur Frage der Keimungshemmenden Substanzen in fleischigen Früchten. Beih. Bot. Centralbl. A. **55**: 191–196. 1936.

76. ————. Albert der Grosse, der Entdecker der Keimungshemmenden Wirkung des Fleisches saftiger Früchte. Zeits. Ges. Nat. **9**: 1936.

77. Kolle, W., Laubenheimer, K. and Vollmar, H. Ueber die oligodynamische Einwirkung dampfförmiger Stoffe auf Gewebekulturen und Bakterienkulturen. Wiss. Woche Frankf **3**: 1–13. 1935.

78. Konis, E. On the inhibiting substances in the tomato fruit. M.S. Thesis, Hebrew Univ. 1938. [Hebrew].

79. ————. On germination inhibitors. II. On the action of germination inhibiting substances in the tomato fruit. Pal. Jour. Bot. Jerus. Ser. II, 6–27. 1940.

80. ————. On germination inhibitors. VI. The inhibiting action of leaf saps on germination and growth. Pal. Jour. Bot., Jerus. Ser. IV. 1947.

81. Koshimizu, T. On the relation between the ripening stages of the maize seed and its germination. Bot. Mag. Tokyo **50**: 504–513. 1936.

82. Kuhn, R., Jerchel, D., Moewus, F. and Moeller, E. F. Über die chemische Natur der Blastokoline und ihre Einwirkung auf keimende Samen, Pollenkörner, Hefen, Bakterien, Epithelgewebe und Fibroblasten. Naturwiss. **31**: 468. 1943.

83. Laer, M. van, Fröschel, R. and Vanderschneren, I. Les substances freinantes de l'orge. Comm. Tech. Inst. Nat. Ind. Ferment. Bruxelles **9**: 1–5. 1942.

84. Laibach, F. and Meyer, F. Über die Schwankungen des Auxingehaltes bei *Zea Mays* and *Helianthus annuus* im Verlauf der Ontogenese, Senckenbergiana **17**: 73. 1935.

85. Laibach, F. und Keil, I. Ueber die Keimungshemmende Wirkung der freien Blausäure. Ber. Deut. Bot. Ges. **55**: 579–583. 1937.

86. Landau, N. The effect of hetero-auxin on the germination of some seeds. Pal. Jour. Bot. Jerus. Ser I, 409–413. 1940.

87. Larsen, P. Ueber Hemmung des Streckungswachstums durch natürlich vorkommende aetherloesliche Stoffe. Planta **30**: 160–167. 1939.

88. ——————. Avena curvatures produced by mixtures of growth promoting and growth retarding substances. Am. Jour. Bot. **34:** 349–356. 1947.

89. Lavialle, P. Observations et considérations sur la germination accidentelle des graines au rein des fruits charnus. Bull. Soc. Bot. France **76:** 276–279. 1929.

90. Lehmann, E. and Aichele, F. Keimungsphysiologie der Gräser. 1931.

91. Lehmann, W. Zur Keimungsphysiologie des Buchweizens. Landw. Jahrb. **84:** 741–778. 1937.

92. Linser, A. Ueber das Vorkommen von Hemmstoff in Pflanzenextrakten. sowie über das Verhältniss von Wuchsstoffgehalt und Wuchsstoffabgabe bei Pflanzen oder Pflanzenteilen. Planta **31:** 32–59. 1940.

93. Litvinov, L. S. On the causes of the inhibiting effect of tomato sap on the germination of tomato seeds. Perm. Biol. Nauch. Issled. Inst. Bull. **11:** 163–171. 1938.

94. Lucas, E. H., Lewis, R. W. and Sell, H. M. An antibiotic principle derived from seeds of *Brassica oleracea.* Mich. Agr. Exp. Sta., Quart. Bull. **29:** 1–3. 1946.

95. Lundegardh, H. Höhere Pflanzen in ihrer Einwirkung auf den Boden. *In* Blanck's Handbuch der Bodenlehre, Vol. 7. 1931.

96. Lustig, B. und Wachtel, H. Ueber biochemische Beeinflussung der Keimung und des Wachstums der Cardaminesamen. I. Mitt. Einfluss der äusseren Faktoren und der Salze. Biochem. Zeits. **296:** 13–27. 1938.

97. Magnus, W. Hemmungsstoffe und falsche Keimung. Ber. Deut. Bot. Ges. **38:** 19–26. 1920.

98. McKnight, R. S. and Lindegren, C. C. Bacteriocidal effects of vapors from crushed garlic on *Mycobacterium Cepae.* Proc. Soc. Exp. Biol. & Med. **35:** 477–479. 1936.

99. Massart, J. Pourquoi les graines ne germent pas dans les fruits charnus. Rec. Inst. Bot. Léo Errera **10:** 83. 1922.

100. Mazé, P. Recherches sur la role de l'oxygène dans la germination. Ann. Inst. Pasteur **14:** 350–368. 1900.

101. ——————. Maturation provoquée des graines. Action antigerminative de l'aldehyde éthylique. Comp. Rend. Acad. Sci., Paris **151:** 1282–1286. 1910.

102. Medawar, P. B., Robinson, G. M. and Robinson, R. A synthetic differential growth inhibitor. Nature **151:** 195. 1943.

103. Merkenschleger, F. Keimungsphysiologische Probleme. Naturwiss. u. Landw. **1:** 1924.

104. Metzner, P. Über die Abgabe fluorescierender Stoffe durch quellende Samen und Früchte. Biochem. Zeits. **224:** 448–458. 1930.

105. Meyer, F. Über die Verteilung des Wuchsstoffes in der Pflanze während ihrer Entwicklung. Diss. Frankfurt, 1936.

106. Molisch, H. Der Einfluss einer Pflanze auf die andere, Allelopathie. 1937.

107. Mosheov, G. The influence of the water extract of wheat seeds upon their germination and growth. Pal. Jour. Bot. Jerus. Ser. I. 86–92. 1938.

108. Nielsen, N. Der Wuchsstoffgehalt von Samen verschiedenen Alters. Comp. Rend. Trav. Carlsberg, Phys. **21:** 427. 1936.

109. Nutile, G. E. Inducing dormancy in lettuce seed with coumarin. Pl. Phys. **20:** 433–442. 1945.

110. Oppenheimer, H. Keimungshemmende Substanzen in der Frucht von *Solanum lycopersicum* und in anderen Pflanzen. Sitzb. Wien. Akad. Wiss. Abt. I. **131:** 59–65. 1922.

111. ————. Das Unterbleiben der Keimung in den Behältern der Mutterpflanze. Sitzb. Wien. Akad. Wiss. Abt. I. **131**: 279–311. 1922.

112. Ozorio de Almeida, A. M., Goulart, M. D., Ielpo, M. and Vieira Pinto, A. Estudo da açâo inhibidora de suco de Solanum lycopersicum sobre a germinação de sementes a crescimento de plantas. Rev. Brasil. Biol. **1**: 345–354. 1941.

113. ————, ————, ———— and ————. Estudo da açâo de suco de fruto de Solanum lycopersicum sobre celulas animales. Rev. Brasil. Biol. **1**: 355–359. 1941.

114. Peters, Th. Die Wirkung des Lichtes bei der Keimung der Samen von *Phacelia tanacetifolia*. Ber. Deut. Bot. Ges. **42**: 381–387. 1924.

115. Pryor, D. E., Walker, I. C. and Stakmann, M. A. Toxicity of allylisothiocyanate vapor to certain fungi. Am. Jour. Bot. **27**: 30–38. 1940.

116. Rademacher, B. Ueber den antagonistischen Einfluss von Roggen und Weizen auf Keimung und Entwicklung mancher Unkräuter. Pflanzenbau **17**: 131–143. 1940.

117. Randolph, L. F. and Cox, L. G. Factors influencing the germination of *Iris* seed and the relation of inhibiting substances to embryo dormancy. Proc. Am. Soc. Hort. Sci. **43**: 284–300. 1943.

118. Reinhard, A. W. Zur Frage der Samenkeimung bei *Solanum lycopersicum*. Planta **20**: 792–794. 1933.

119. Reinitzer, F. Ueber Ermüdungsstoffe der Pflanzen. Ber. Deut. Bot. Ges. **11**: 531–537. 1893.

120. Rippel, K. Zur Methodik des quantitativen Nachweisens von Zellteilungshormonen mittels *Saccharomyces cereviseae*. Planta **27**: 38. 1937.

121. Roblin, R. O., Jr. Metabolite antagonists. Chem. Rev. **38**: 255–377. 1946.

122. Rochlin, E. Zur Frage der Widerstandsfähingkeit der Cruciferen gegen Kohlhernie. Phytopath. Zeits. **5**: 381–406. 1933.

123. Ruge, U. Zur Physiologie der genuinen Keimungshemmenden und beschleunigenden Stoffe von Helianthus annuus. Zeits. f. Bot. **33**: 529. 1939.

124. Schmid, H. Notiz über Keimungshemmende Stoffe. Helvet. Chim. Acta **27**: 1197–1199. 1944.

125. Schuck, A. L. A growth inhibiting substance in lettuce seeds. Science **81**: 236. 1935.

126. ————. The formation of a growth inhibiting substance in germinating lettuce seeds. Proc. Int. Seed Test Assoc. **7**: 9–14. 1935.

127. Scott, D. H., Waugh, J. G. and Cullinan, F. P. An injurious effect of peach juice on germination of the seed. Am. Soc. Hort. Sci. **40**: 283–285. 1942.

128. Sigmund, W. Über die Einwirkung von Stoffwechselendprodukten auf die Pflanzen. I. Einwirkung N-haltiger Stoffwechselendprodukte auf die Keimung von Samen (Alkaloide). Biochem. Zeits. **62**: 299–338. 1914.

129. ————. Über die Einwirkung von Stoffwechselendprodukten auf die Pflanzen. II. Einwirkung N-freier pflanzlicher Stoffwechselendprodukte auf die Keimung von Samen (Glucoside, Gerbstoffe und ihre Spaltungsprodukte. Biochem. Zeits. **62**: 339–386. 1914.

130. ————. Über die Einwirkung von Stoffwechselendprodukten auf die Pflanzen. III. Einwirkung N-freier pflanzlicher Stoffwechselendprodukte auf die Keimung von Samen (Aetherische Oele, Terpene u. a.). Biochem. Zeits. **146**: 389–419. 1924.

131. Sinclair, W. B. and Eny, D. M. The organic acids of lemon fruits. Bot. Gaz. **107**: 231–242. 1945.

132. Slizynsky, B. M. Production of structural changes in somatic chromosomes of *Drosophila melangaster.* Nature 159: 66–67. 1947.

133. Sroelov, R. On germination inhibitors. IV. Germination inhibitors of *Sinapis alba* and other seeds when enclosed in their fruit. Pal. Jour. Bot. Jerus. Ser. II: 33–37. 1940.

134. Stewart, W. S. A plant growth inhibitor and plant growth inhibition. Bot. Gaz. 101: 91–108. 1939.

135. Stout, M. and Tolman, B. Factors affecting the germination of sugar beet and other seeds with special reference to the toxic effects of ammonia. Jour. Agr. Res. 63: 687–713. 1941.

136. ———— and ————. Interference of ammonia released from sugar beet seed balls with laboratory germination tests. Jour. Am. Soc. Agron. 33: 65–69. 1941.

137. Teodoresco, M. E. L'athermanéité des essences végétales et la transpiration. Rév. Gén. Bot. 35: 1–45. 1923.

138. Tetjurew, W. A. Über die Keimungshemmende Substanz in saftigen Früchten. Jour. Bot. (U.S.S.R.) 1938.

139. ————. Über das sogenannte "Blastokolin". Planta 32: 211–226. 1941.

140. Thorton, N. C. Importance of oxygen supply in secondary dormancy and its relation to the inhibiting mechanism regulating dormancy. Contr. Boyce Thompson Inst. 13: 487–500. 1943/45.

141. Tilly, F. Über Sensibilisierung und Desensibilisierung lichtempfindlicher Samen (*Lythrum Salicaria* L.). Zeits. Bot. 28: 401–445. 1934/35.

142. Tokin, B. Phytoncides. *In* The Progress of Soviet Medicine during the War. I. Microbiology and Epidemiology. Narkomzdrav U.S.S.R. Medgis 1943 [Russ.].

143. ————. Effect of phytoncides upon protozoa. Am. Rev. Sov. Med. 1: 237–239. 1944.

144. Tolman, B. and Stout, M. Toxic effect on germinating sugar beet seed of water soluble substances in the seed ball. Jour. Agr. Res. 61: 817–830. 1940.

145. Toni, G. B. de and Mack, P. Sopra l'influenza esercita dalla nicotina e dalla solanina sulla germogliazione dei semi di tobacco. Parma: 63–68. 1893.

146. Toroptsev, I. V. and Filatova, A. G. The use of phytoncides in treatment of infected wounds. Am. Rev. Sov. Med. 1: 244–250. 1944.

147. Tschirch, A. Handbuch der Pharmakognosie. 1923.

148. Ullman, S. B. On germination inhibitors. V. Essential oils, alkaloids and glucosides as inhibitors of germination and growth. Ph.D. Thesis, Jerusalem, 1940.

149. Vahl, I. On germination inhibitors. III. Germination inhibitors in the fruit of *Poterium spinosum* L. Pal. Jour. Bot. Jerus. Ser. II: 28–32. 1940.

150. Veh, R. v. Eine neue Methode der Anzucht von Sämlingen unabhängig von Ruheperiode und Jahreszeit. Züchter 8: 145–151. 1936.

151. ———— und Söding, H. Wuchsstoff und Keimung der Obstbaumkerne. Ber. Deut. Bot Ges 55: 270–278. 1937.

152. Veldstra, H. Researches on plant growth substances. IV. Relation between chemical structure and physiological activity. I, II. Enzymologia 11: 97–163. 1943/45.

153. ———— and E. Havinga. On the physiological activity of unsaturated lactones. Enzymologia 11: 373–380. 1943/45.

154. Voss, H. Wuchsstoffaktivierung und inaktivierung und ihre Keimungsregulatorische Bedeutung. Planta 27: 432–435. 1939.

155. ————. Nachweiss des inaktiven Wuchsstoffes, eines Wuchsstoffantagonisten und deren Wachstumregulatorische Bedeutung. Planta 30: 252–285. 1939.

156. Waks, C. The influence of extract from *Robinia pseudacacia* on the growth of barley. Publ. Fac. Sci. Univ. Charles, Prague **150**: 84–85. 1936.

157. Walker, I. C., Morell, S. and Foster, H. H. Toxicity of mustard oils and related sulphur compounds to certain fungi. Am. Jour. Bot. **24**: 536–541. 1937.

158. Wallenfels, K. Symbiose und Antibiose. Chemie **58**: 1–16. 1945.

159. Walton, L., Herbold, M. and Lindegren, C. C. Bactericidal effects of vapors from crushed garlic. Food Res. **1**: 163–169. 1936.

160. Wehmer, C. Versuche über Blausäurewirkung auf Pflanzen. Biochem. Zeits. **92**: 364–375. 1918.

161. Wiesner, I. Vergleichende physiologische Studien über die Keimung europäischer und tropischer Arten von *Viscum* und *Loranthus*. Sitzber. Akad. Wiss. Wien, Math.-Nat. Kl. 1 Abt. **103**: 401–437.

162. ———. Ueber die Ruheperiode und über einige Keimungsbedingungen der Samen von *Viscum album*. Ber. Deut. Bot. Ges. **15**: 503–516. 1897.

163. Zimmermann, P. W. and Hitchcock, A. E. Activation of cinnamic acid by ultra violet light and the physiological activity of its emanation. Contr. Boyce Thomps. Inst. **10**: 197–200. 1938/39.

164. Zlataroff, A. Ueber das Altern der Pflanzen. Zeit. Allg. Phys. **22**: 1916.

165. Zohary, M. Die verbreitungsökologischen Verhältnisse der Pflanzen Palästinas. Beih. Bot. Centralbl. A. **56**: 1–155. 1937.

ADDITION

After the manuscript was already in print the papers cited below have been published or came to my knowledge.

Based on the observation of Went (179) that some desert shrubs favour the growth of certain annuals whereas annuals very rarely are found in the vicinity of *Encelia farinosa*, Gray and Bonner (169) isolated a growth inhibitor from the leaves of this plant which is 3-aldehydo-4-methoxy acetophenone. This toxic substance may be leached out from the fallen *Encelia* leaves and cause inhibition of germination of seeds in the vicinity of *Encelia* plants.

The importance of inhibitors for plant sociology and agriculture was stressed already in 1907 (174). Schreiner and co-workers (174–178) report that many soils are unproductive because of the presence of toxic substances which in some cases are excreted by roots. From soil which by constant recropping with cowpeas was made infertile for this crop a substance could be extracted which inhibited the growth of cowpeas in the water culture (177).

This offers an explanation for the well known phenomenon of soil fatigue. The same author has been the first to report that cinnamic acid and coumarin are strong growth inhibitors (175).

Pickering (171, 172) formulated long before Molisch (106) the idea of "allelopathie" by stating that "the deleterious effect of one growing plant on another is a general phenomenon" (171), and explains by toxin production the well known baleful effect of grass on fruit trees.

The bark of peach roots has to be added to the list of plant organs containing inhibitors (173). Hydrogen cyanide produced from amygdalin is most probably the inhibiting agent.

The toxic effect of *Juglans nigra* trees on certain plant associations is explained by excretion of juglone (5-hydroxy alpha-napthaquinone) (167).

Human saliva was reported to exert a germination inhibiting influence (180).

166. Benedict, H. M. The inhibitory effect of dead roots on the growth of bromegrass. Jour. Am. Soc. Agron. **33**: 1108–1109. 1941.
167. Davis, E. F. The toxic principle of *Juglans nigra* as identified with synthetic juglone and its toxic effect on tomato and alfalfa plants. Am. Jour. Bot. **15**: 620. 1928.
168. Duke of Bedford and Pickering, S. U. Effect of one crop upon another. Jour. Agric. Sci. **6**: 136–151. 1914.
169. Gray, R. and Bonner, J. An inhibitor of plant growth from the leaves of *Encelia farinosa*. Am. Jour. Bot. **35**: 52–57. 1948.
170. Myers, M. E. and Anderson, K. Bromegrass toxicity vs. nitrogen starvation. Jour. Am. Soc. Agron. **34**: 770–773. 1942.
171. Pickering, S. The effect of one plant on another. Ann. Bot. **31**: 181–187. 1917.
172. ————. The action of one crop on another. Jour. Roy. Hort. Soc. **43**: 372–380. 1919.
173. Proebsting, E. L. and Gilmore, A. E. The relation of peach root toxicity to the re-establishing of peach orchards. Proc. Am. Soc. Hort. Sci. **38**: 21–26. 1940.
174. Schreiner, O. and Reed, H. S. Some factors influencing soil fertility. Bull. U. S. Dept. Agric. Bur Soils **40**: 1907.
175. ———— and ————. The toxic action of certain organic plant constituents. Bot. Gaz. **45**: 73–102. 1908.
176. ———— and Skinner, J. J. Certain organic constituents of soil in relation to soil fertility. Bull. U. S. Dept. Agric. Bur. Soils **47**: 1907.
177. ———— and Sullivan, M. X. Soil fatigue caused by organic compounds. Jour. Biol. Chem. **6**: 39–50. 1909.
178. ———— and Shorey, E. C. The isolation of harmful organic substances from soils. Bull. U. S. Dept. Agric. Bur. Soils **53**: 1909.
179. Went, F. W. The dependence of certain annual plants on shrubs in Southern California deserts. Bull. Torrey Bot. Club **69**: 100–114. 1942.
180. Yardeni, D. Human saliva as a germination inhibitor. Sci. **108**: 62–63. 1948.

EFFECTS OF MINOR ELEMENTS ON THE PHYSIOLOGY OF FUNGI

D. PERLMAN[1]

INTRODUCTION

Numerous reports on the effects of metallic ions on the physiology of fungi have appeared during the nine year period (1939–47) since this subject was reviewed by Foster (41). It is evident that metallic ions play important roles in the production of many chemicals including the antibiotic substances by microorganisms, and some advance has been made in understanding the effects of metallic ions on fungal metabolism as a by-product of the recent intensive research on the mycological production of these compounds. However, there is still considerable ground to be covered before the specific roles of these metallic ions in the metabolic functions of the organisms is fully understood.

It has been decided to confine the material covered in this review nearly entirely to the heavy metals and to omit many of the studies concerned with the effects of the alkaline earth group. In order to integrate the literature in a satisfactory manner, it will be reviewed by organisms rather than by individual elements. While this method does not allow comparisons to be easily drawn, it seems more reasonable, since experimental conditions differ greatly in different laboratories, and generalizations are thus of somewhat limited value. Inasmuch as the actinomycetes apparently resemble fungi in the order of their requirements and their responses to the presence of metallic ions in the medium, they will be included in this discussion.[2] Neuberg (100) has included a general discussion on recent developments on the effects of metallic ions on yeast growth and metabolism, and more specific information is mentioned by Joslyn (59). Schopfer (128) and Stiles (150) have discussed the general subject of metallic ions with regard to the physiology of fungi in their recent books.

---

[1] Present address: 35 Edgehill St., Princeton, N. J.

[2] These organisms will not be included in a review on the effects of metallic ions on bacterial physiology (Dr. S. G. Knight, personal communication).

PURIFICATION OF MEDIA

In order to study the effects of specific ions on the physiology of an organism, it is advisable to have media which contain only known constituents. Most synthetic media contain sufficient contaminating quantities of these minor elements to be biologically important, and natural media have an abundance of them. It is necessary to remove these contaminants without affecting the other constituents of the medium. A number of methods have been proposed or have been rather widely used during the past few years and will be mentioned. Unfortunately it is not often possible to gauge the effectiveness of these methods in reducing the metallic content of the medium, since the quantities present before and after treatment are rarely stated. Stout and Arnon (153) have discussed methods for the purification of a number of medium constituents and criteria of purity.

Among the chemical methods recently described is that in which 8-hydroxyquinoline is used to form complexes with metallic ions (164, 175). Other related compounds may also be used (4, 5, 6, 19). The complex as well as the residual reagent is often removed from the medium by chloroform extraction before sterilization and inoculation. Other complex-forming agents and precipitants include ferrocyanide, ferricyanide (89) and calcium sulfide (20) which have been used in the purification of media in which molasses is a constituent (14, 15, 49, 68, 108, 135). Hutner (52, 53) has suggested the addition of certain hydroxy-organic acids to the medium to form complexes with metallic ions and apparently make them unavailable to microorganisms. This may also occur when organic acids, *e.g.*, citric, oxalic, tartic, are formed during the fermentation (111), and thus limit the effect of specific ions during fermentations. As can be seen this becomes of practical importance when such fermentations are operated on a large scale with several intermediate fermentation steps. The addition of peroxide (87) and other reagents (76) may also accomplish this biologically unavailable complex formation. Other specific relationships also have been reported, including histidine-cobalt (26, 110).

Treatment of the medium with cation-exchange materials has been suggested as a non-selective method of removing contaminating elements. Of course, this method is applicable only to the non-ionic portion of the medium, and usually inorganic salts are added after treatment. The method is quite effective in reducing the quantities of metallic ions present (106, 165). It has been applied to synthetic media (105, 109, 110) as well as molasses solutions (60, 62, 117, 118, 135, 163, 169).

A practical method giving results similar to those obtained with the cation-exchange material was recently described by Shu and and Johnson (133b) who used aluminum hydroxide as a coprecipitating agent.

OBSERVATIONS ON THE EFFECTS OF METALLIC IONS ON GROWTH AND METABOLISM OF FUNGI

Ashbya gossypii. Wickerham *et al.* (166) have noted that an orange-yellow variant produced high yields of riboflavin when grown on unpurified media containing Fe.

Aspergillus clavatus. It has been observed that the use of raw sugar in place of technical glucose resulted in somewhat greater antibiotic potency (presumably patulin), and Waksman *et al.* (162) comment, "This suggests the possible function of some active agent or trace element present in the raw sugar".

Aspergillus flavus. Copper is apparently needed for growth and spore production (93). The poisonous effects of high concentrations of $MgSO_4$ on growth were counteracted by $HgCl_2$ (120). Pontovich (113) mentions that U radiation of this organism growing in a medium deficient in K showed that U prevented formation of melanins and stimulated riboflavin production.

Aspergillus fumigatus. Menzel *et al.* (88) found that the addition of Zn to Czapek-Dox medium completely suppressed the production of gliotoxin without affecting mold growth, and resulted in the formation of a red pigment. The reduced antibiotic activity noted when tap water was used in the prepartion of the medium may have been due to the inorganic contaminants. Waksman *et al.* (162) reported that the addition of Zn to media depressed production of fumigacin (a mixture of gliotoxin and helvolic acid) and appeared to favor its rapid destruction.

Aspergillus glaucus. Copper has been found essential for growth and spore production (93).

Aspergillus niger. The interest in the effects of metallic ions on the growth of this organism has continued during the past decade.

Steinberg's reports have dealt with effects of certain ions on the growth at 35° C. of a certain strain of *A. niger* on a sucrose-salts medium. His previous studies (see 41) have indicated the essentiality of Fe, Cu, Zn, Mn, Mo and Ga for this strain grown under these conditions. Mo seems to be of importance in reduction of NO_3, NO_2 and hydroxylamic acid (139), while all of the group are essential for maximum growth on media containing either amino acids or inorganic nitrogen (142, 144). He reports (140) that the source of carbohydrate does not influence the trace element requirements, and sucrose, glucose, fructose, mannose and sorbose all served equally well, while Sc (in addition to the other six) increased growth on gylcerol. A later investigation (145) confirmed these observations and indicated that the trace element requirements for maximum growth on media containing monoses as source of carbohydrate were simiiar to those with sucrose. The above mentioned six elements were required in approximately equal degrees, whatever state of oxidation of sulfur was supplied as nutrient (143). and omission of these six when CO_2 was absent from the atmosphere above the mycelium resulted in a greater reduction in growth than when CO_2 was present (146). In studies with a non-optimal medium increased growth was obtained when Ti, Cb and Pd were added (148), and Na and Be increased growth when the medium was deficient in K and Mg (149).

Other investigators have used other strains of *A. niger* and other laboratory conditions and media, and it is not surprising that results are often somewhat contradictory. Bertrand has observed (11, 12, 13) that the addition of V to medium containing Fe, Zn, Cu, Mn and Mo increased growth markedly. When starch was the carbohydrate source another investigator found that Zn, Cu, Fe and Mn were necessary for optimum growth (40). Zn has been reported as necessary for the utilization of glucose (for growth) from Raulin's medium (63) and the formation of cellulose (64). Neilsen (103) has summarized his observations by suggesting that mixtures of Cd, Ba, Sr, Ca, Li, Zn, Be, Cu and Mn in small quantities act as a co-growth substance. Mo is apparently necessary for maximum growth on a medium containing arabogalactan (116). Many have noted that *A. niger* will grow under what normally would be considered extreme conditions; an example is the report that a culture grew on media containing 0.3 M in Al (22).

In a recent review (147) Steinberg has concluded that the mineral requirements of *A. niger* agree with those of green plants, and thus the use of this organism in testing soil samples has been understandably widespread. Spore color in these tests is associated with the presence of available Cu (2), and while Zn is also essential for growth it cannot replace Cu (58). The presence of Mn in soil samples tested by this procedure resulted in increased growth, while Al and Ti when present often had a stimulatory effect (104). Sporulation was reduced when Fe, Mo or Mn was absent, but not when Cu, Zn or Ga was missing (148). The *A. niger* growth test has been suggested for the qualitative testing of blood for the presence of Fe and Zn (95), as well as the testing of soil for available Mg (136). Zn, Fe and Cu are not necessary for spore germination (65).

Riboflavin production by *A. niger* mentioned in the previous review has been investigated further. While stimulation has been reported upon the addition of Hg to the medium (69), this has not been confirmed (27), and another report indicates that addition of $HgCl_2$ results in release of riboflavin into the medium (70). Traces of minerals present in tap water were unfavorable to production of flavin type pigments (73).

It is usually assumed that metallic ions exert their effects on specific enzyme systems, although concrete evidence on the mode of action is often lacking. Hydrolysis of arabogalactan was increased when Cu, Fe, Zn, Mn and Mo were added to the medium (116), and when the first four of this group were added to a starch medium, growth was increased, but amylase and starch decomposition were reduced (40). Zinc has been reported to be necessary for the utilization of glucose in Raulin's medium (63). Addition of Ra to a medium resulted in affected growth and subsequent production of amylase and proteinase (96, 97, 98). It seems likely that the effects of certain organic substances may be traced to their metallic ion impurities (141) and not to specific effects on enzyme systems.

Several organic acids of economic importance are produced by this organism, and it is not surprisng to find that metallic ions affect the production of these chemicals. Several reports have indicated effects on total acid production only and have not mentioned specific acids. These include mention that addition of iron to Raulin's medium results in initial acid production and subsequent utilization of this acid (54) probably similar to observations on citric acid production (111). Another report indicated that added Zn and Fe had no effect on acid production from raw cane sugar (174).

Gluconic acid production was thought to be reduced by iron dissolved from the fermentation vessel (114), and in this connection the inhibitory effect of various metals often used for construction of fermentation vessels on citric acid production is of interest (135). In the latter report stainless steel strips placed in fermentation flasks resulted in reduced yields, while aluminum strips did not. It has been reported that the addition of Hg to a glucose or sucrose medium shifted the fermentation from a citric-oxalic type to gluconic acid (69).

Citric acid production in both surface and submerged culture has been investigated in a number of laboratories. Impurities in ash of beet sugar (9) and molasses (68, 105, 108) markedly affected production. Ferrocyanide treatment of molasses removed some of the inhibiting materials (14, 15, 49, 68, 108, 135).

When synthetic media have been used in surface culture, yields of citric acid and effects of metallic ions on citric acid production have varied with the strain of *A. niger* used. Bernhauer *et al.* (10) report that Mn had no consistent effect on citric acid production, and Sn, Al, Co, Ni, Cr, Se, Pd, Pt, Cu and Fe (all at 0.01%) had no effect, while Zn, Ag, Te, U, Sb and W inhibited citric acid production by a certain strain of *A. niger*. Other strains have been stimulated by the addition of Fe, Mn, Cr and Al (105, 109), although mixtures of these elements were not better than the single elements, and the optimum concentration varied from one strain to another. This latter qualification seems general, for the stimulatory effect of Se is reported to vary with the culture as well as the purity of the sugar (74). As might be expected from the foregoing, some cultures are more affected by impurities in the medium (usually from the water source) than others (75).

When a medium in which a treated beet molasses was used as source of fermentable carbohydrate, citric acid production (in surface culture) was reduced by the addition of salts of a number of elements, including Fe, Zn, Pb, Mn and Cu (68), as well as ash from certain untreated molasses (108).

In submerged culture the addition of Mn markedly reduced citric acid production by one strain of *A. niger*, although it had no effect on citric acid production in surface culture by this same strain (132, 133) and little effect in submerged culture by other strains (111). Fe was definitely necessary for maximum yields of acid (111, 132) when grown on a synthetic medium. Some strains require less Fe than others, and when high concentrations are added, yields are markedly reduced by these strains. On the other hand, other strains are relatively tolerant of high Fe levels (111). Mn, Zn, Al, Cr and other ions also affect production (60, 111). It has been shown that addition of Mn to the sporulation medium reduces the yield of citric acid produced when these spores are used as inoculum in fermenters (133), and that Fe and Cu did not exert this effect. Shu and Johnson (133a, 133b) have studied the effects of Fe, Zn, $MgSO_4$ and KH_2PO_4 on the production of citric acid and conclude that these factors are interdependent.

Aspergillus oryzae. Yoshimura (173) has studied the effect of metallic ions on the production of catalase by this organism. Addition of Fe, Zn, Cu or Mn at low concentrations to the medium favored catalase production, while higher concentrations of the latter two inhibited spherical cell formation and accelerated conidial production which generally counteracted catalase activity.

Aspergillus terreus. Production of itaconic acid by strains of this organism has been investigated in surface and submerged culture. Lockwood and Reeves (84) report that the addition of Mn, Mo, Co, Cu, Ni, Cr and Ga to the media containing salts and technical grade glucose had no effect on acid production. The inhibitory effect of Al was counteracted by the addition of $MgSO_4$. The stimulation of acid production on the addition of Fe and Zn depended on the pH of the medium. Moyer and Coghill (90) used another strain of this organism and found that the addition of Fe and Zn to a purified medium containing salts and glucose resulted in increased acid production. Better results were obtained when the medium contained technical glucose than with CP glucose or sucrose. Mixtures of Cb and Zn, or Mo and Zn, were apparently equivalent to Fe and Zn mixtures in increasing acid production, and these investigators concluded that "with the possible exception of Zn there appears to be little gain from the use of trace elements." In submerged culture the addition of Zn to a salts-glucose-corn-steep water medium resulted in no appreciable change in acid production (85).

Aspergillus wentii. Karow and Waksman (60, 61, 62, 163) have studied the production of citric acid in submerged culture by this organism, and conclude "the concentration of nutrients must be restricted to a minimum; barely sufficient to allow good mycelial development and not so high as to permit maximum growth of the organism." Highest yields of citric acid were obtained when small amounts of Mn and Zn were added to the medium. Addition of higher concentrations of Zn or Fe did not favor acid production, and certain levels of Mn favored pigment production and mycelial growth.

Aspergillus sp. Timmonin and Rouatt (158) and Wyllie (172) have studied the production of citrinin by a culture of *Aspergillus* of the *glaucus* group when grown on media containing glucose, fructose or sucrose and inorganic salts. Production was increased when Fe and Zn were added to the medium. Maple sugar was a better carbohydrate source than sucrose, perhaps because of its metallic ion content.

Cephalosporium sp. Starkey (137) isolated a culture of this organism and one of *Acontium velatum* growing in saturated $CuSO_4$ solution at pH 0.2.

Epidermophyton floccosum. The inhibitory effect of Zn on oxygen uptake (101) could be reversed by addition of Ca, $MgSO_4$ or $Na_2S_2O_3$ (102).

Fusarium graminearum. The production of an orange-yellow pigment on Czapek-Dox medium was increased by addition of Mn and Zn to the medium (94).

Fusarium hyperoxysporum. Texera (154) reports that addition of Zn to Czapek-Dox medium is necessary if two antibiotics are to be produced by this organism. If Zn is absent the glucose fermentation is incomplete and only the antibiotic present in the mycelium is produced. Mn and Cu had no effect on the elaboration of these substances.

Fusarium oxysporum. Zentmeyer (175) reported that Zn reversed the fungistatic action of 8-hydroxyquinoline towards this organism, and suggested that Zn is the most important trace element for its growth.

Memnoniella echinata. Addition of Fe, Mn or Zn to a salts-glucose medium resulted in increased mycelial development, while traces of Ni, Co, Cr or Al decreased growth (110). The addition of histidine reversed the inhibitory effect of Co (110), as suggested by the previous studies by Burk *et al.* (26), on the combinations of histidine and Co.

Monosporium sp. Bojanovsky (21) found that organisms isolated from soils grew best on media containing added Fe.

Mucor pusillus. The growth-inhibiting effects of high levels of $MgSO_4$ can be reversed by the addition of $HgCl_2$ (120).

Mucor ramannianus. A symbiotic relationship between this organism and *Rhodotorula rubra* was found in media containing aspargine for thiamine. When other nitrogen sources were used Li, Co, Mo, Hg, U and Al were necessary additions to the medium (159).

Neurospora crassa and *N. sitophila.* The addition of a trace element mixture containing Zn, B, Mo, Mn, Fe and Cu to a number of nutrient solutions all containing Fe increased the growth rate slightly; the tartrate used in this medium was found to be contaminated with trace elements (125). In liquid culture traces of Zn have been found to be stimulatory to growth (23, 28, 152) and to repress sporulation (151).

Penicillium brevicaule. Bird and Challenger (16, 17) have continued their studies on methylation with this organism and report that addition of K_2TeO_3 to Czapek-Dox medium results in the formation of $(CH_3)_2Te$. The addition of sodium salts of R SeO_2H (R: CH_3, CH_3CH_2, or $CH_3CH_2CH_2$) resulted in formation of the mixed and di selenides of these precursors.

Penicillium chrysogenum. Recent interest in the effect of metallic ions on the growth and metabolism of this organism stems mainly from its role in the production of penicillin. It is probable that many of the effects noted will be observed in the closely related *P. notatum.* Jarvis and Johnson (55, 56, 57) have reported that

Zn, Fe and Mn were essential for optimum growth and penicillin production on a synthetic medium. Mn was necessary when a purified medium was used, as was Ca, but these two were not essential when a less pure media (U.S.P. ingredients) were used. They found no requirement for Cr, which had been reported (115) as stimulatory to penicillin yields. Pratt and Dufrenoy (115) could not replace Al and Cr by Mo, Co or Ni. Knight (71) and Koffler *et al.* (78) have tested a large number of the constituents of cornsteep liquor ash which had been found to stimulate penicillin production (72, 77), including Al, As, Cr, Co, Cu, Fe, Pb, Mn, Ni, Ag, Sn, W and Zn. The combination of Fe and phosphate was as effective as cornsteep ash in promoting penicillin production. Fe and Cr combinations also increased penicillin production, while Al antagonized the stimulatory effect of Fe. The presence of more than 2 ppm of Cu prevented accumulation of penicillin, but this effect was nullified by the addition of Fe. Woodruff (168) has commented that the heavy metal concentration of the medium had little effect on the differential bioassay (*Staphylococcus aurcus*—*Bacillus subtilis* comparative response), although the final antibiotic potency of the broth was altered.

Other observations with this organism include that of Moyer (91) that the yield of gluconic acid was reduced when Zn was added to a glucose-salts-cornsteep liquor medium, and that of Bird and Challenger (16) that $(CH_3)_2Te$ and $(CH_3)_2Se$ were evolved when K_2TeO_3 or Na_2SeO_3, respectively, were added to Czapek-Dox medium or bread cultures.

Penicillium citrinum. Bailey and Cavallito (7) noted a 30-fold increase in the yield of citrinin when Fe was added to salts-glucose medium. Other studies (82) have shown that the Fe may be replaced by Mn but not by Cu or Zn.

Penicillium notatum. The recent studies on penicillin production by this organism have included several observations on the role of metallic ions in the biosynthesis of this substance. It is probable that many of the reports dealing with this organism apply equally to the closely related *P. chrysogenum.* In their early paper the Oxford group (1) noted that the use of tap water instead of distilled water did not result in any change in penicillin yield. Other studies with this organism in surface culture (30) seemed to indicate that the addition of Cu increased penicillin yield; while this effect was difficult to repeat, it was noted that pigment production was lower in media containing additional Cu. Other studies (surface culture) by Cook *et al.* (31, 32, 33) indicated that the addition of pea extract increased penicillin production, and that part of the stimulation could be traced to the presence of metallic ions in the extract ash. Foster *et al.* (45) noted that the addition of Zn to a glycerol-salts medium increased penicillin production in the presence or absence of Fe, Mn or Cu. He also noted that some of the superiority of brown sugar media over those containing purified sugars could be found in the ash of the brown sugar. Moyer and Coghill (91) report that *P. notatum* showed a typical trace element requirement in a purified synthetic medium. They added a large number of elements, including Zn, Fe, Cu, Mo, Mn and Cb, to a synthetic medium, and found that only the addition of Zn of this group resulted in increased penicillin yield and also growth. The production of gluconic acid during the penicillin production phase on this medium was reduced when Zn was added to the medium. On the other hand, Negroni (99), studying this problem with another culture, has reported that the addition of Zn to a synthetic medium containing starch had no effect on penicillin production in surface culture.

When submerged culture methods were used, somewhat different results were observed. However, as a different strain was usually used in submerged culture, some of these observations may be the result of strain differences. Foster *et al.* (46) tested the effect of the addition of a number of elements to a brown sugar-salts medium on penicillin production and found that better yields of penicillin were obtained when Zn was added to the medium. He observed no apparent effect when Ca, Fe, Mn, Cu, Cd, Mo or W was added. Moyer and Coghill (92) found that the addition of Mn or Zn to a medium containing cornsteep liquor had no effect on penicillin production. Perlman (107) added Fe, Cr, Mn, Al, Sn and Cu to a synthetic medium and noted that when any one of the first five was added yields of penicillin were reduced. Stefaniak *et al.* (138) report that metal toxicity experiments indicated that aluminum and allegheny metal were nontoxic, while iron exhibited slight toxicity. A total iron content of 500 ppm or more reduced yields of penicillin in their laboratory. Gilbert and Hickey (50) have stated that the addition of appreciable quantities of iron to a cornsteep medium caused decrease in penicillin production and increase in conidia formation. Foster *et al.* (43) mention that heavy metals affect conidiospore formation in submerged cultures by this organism.

Notatin (glucose aerodehydrogenase) production by this organism was reduced when Zn was added to the medium (35), and addition of Cu and Fe was necessary for maximum production of this enzyme. Impurities in brown sugar were responsible for increased production of notatin, according to other investigators (122).

Bird and Challenger *et al.* (16) have noted that this organism produces $(CH_3)_2Te$ and $(CH_3)_2Se$ when the respective salts were added to media, as had been observed with *P. chrysogenum.*

Penicillium patulum. Birkinshaw *et al.* (18, 19) have reported that this organism will produce patulin and gentisyl alcohol when grown on Raulin-Thom medium. Brack (24) has studied this further and observed that increasing the quantity of iron in the medium resulted in increased production of gentisyl alcohol and reduced production of patulin. Cu in presence of Fe reduced production of both substances and increased sporulation, while Zn in presence of Fe increased gentisyl alcohol and reduced sporulation. The addition of Mn to the medium containing Fe increased sporulation, eliminated production of gentisyl alcohol and increased patulin yield.

Penicillium phoeniceum. Curtin *et al.* (36) observed that the addition of Zn to a synthetic medium reduced pigment production.

Penicillium terlikowskii. The increase in gliotoxin production on media containing impure sugar, as compared with media containing purified sugar, was attributed to impurities, probably inorganic in nature (25).

Penicillium waksmanii. This organism is reported (51) to be able to grow in a saturated solution of copper sulfate as well as in concentrated solutions of $ZnSO_4$ and $MnCl_2$. Sporulation and spore germination are retarded by even dilute solutions of $CuSO_4$.

Penicillium sp. An apparent association was observed between mold growth and treatment of cut potato tubers with salts of Cu, Co, Ni and Fe (38). Good growth was obtained on tubers moistened with 2.5% Cu and Co salts; less stimulation was noticed when Ni and Fe were used, and only slight stimulation occurred when Hg was used (37).

Zentmeyer (175) noted that when 8-hydroxyquinoline was added to a medium whose pH was above 3.2, growth of a *Penicillium* culture was inhibited, and this effect could be reversed by the addition of Zn.

Phycomyces blakesleeanus. Leonian and Lilly (79) found that the addition of agar ash or Zn to a purified medium containing thiamin, salts, amino acids and glucose doubled growth. The addition of Cu, Ga, Mn, Mo and Fe singly or in combination had no effect. When the medium was purified by the $CaCO_3$ adsorption procedure, a stimulation of growth was noted when Fe was added to the medium. A later report (80) indicated that the addition of Mo, Mn or Ga to a medium containing Fe and Zn had no effect on zygospore formation. Production of biotin by this organism on a similar medium was increased by the addition of a mixture of salts containing Cu, Fe, Mn, Zn, Ga and Mo (127).

Utiger (160) suggests that the metallic ions present in plant material interfere with the bioassay for thiamin, utilizing this organism, as indicated by the discrepancy between the values obtained with the thiachrome method and the *Phycomyces* growth test. Schopfer (129) does not think that Utiger's conclusions were justified.

Phymatotrichum omnivorum. Experiments by Blank (19*a*), studying the effect of the addition of single elements to a basal medium, indicated that the addition of Zn, Mn or Fe to an inorganic salts-glucose medium (purified by the $CaCO_3$ absorption technique) increased mycelium formed, while addition of Al, Cd, Co, Ni or Cu resulted in reduced mycelium growth, and addition of Si or Mo under the same conditions had no effect. He also observed that the addition of Al, Cd, Co, Hg, Mo, Ni or Si to the basal medium containing Mn, Fe and Zn did not result in increased mycelium growth, and concluded that these elements are not essential for optimum growth. These observations had been previously summarized by Rogers (123*a*). Ezekiel's studies (39*a*) on a similar medium indicated that a mixture of Zn, Cu, Mn and Fe increased mycelium growth when added to the basal medium.

Polyporus adustus and *P. abietinus.* Fries (47) did not find that the addition of Zn, Cu and Mn to a glucose-salts (including Fe) medium affected growth.

Polyporus vaporarius. This organism produced enough oxalic acid to reverse the growth-inhibiting effect of $CuSO_4$ present in impregnated timbers (67).

Rhizopus delemar. Erb and Hildebrandt (39) found that the addition of aluminum powder to a mash containing stillage permitted heavy growth. It was first thought that the aluminum counteracted some toxicity due to traces of other metals picked up during mashing or distilling, but this was not a complete explanation, since stillage produced in glass was similarly toxic for the mold until treated with aluminum.

Rhizopus oryzae. Lockwood (83) notes that addition of Zn to a glucose-salts medium resulted in more rapid germination of spores. In submerged culture the Zn did not influence acid production, while it reduced it in surface culture.

Rhizopus suinus. Nielsen (103) has observed that a mixture of metals, including Cd, Ba, Sr, Ca, Li, Be, Zn, Cu and Mn in small quantities, acted as a co-growth substance. Others have deduced that the stimulation of growth found by adding glucose ash (34) or filter paper ash (170, 171), also called cofactor B, was apparently an inorganic salt of metallic ions, perhaps Fe or Zn.

Rhizopus sp. Foster and Waksman (44, 161), as mentioned in the previous review (41), have noted that addition of Zn to the medium results in increased growth and reduced fumaric acid and ethanol accumulation. Zn apparently stimulates the growth and respiratory coefficient of the organism, but is not favorable to the accumulation of lactic acid.

Sclerotium delphinii. While addition of Fe, Zn, Cu, or Mn did not increase growth on a synthetic medium, the addition of Zn or Mn increased color of the sclerotium. Addition of Cr, Al, Co, or Ni resulted in significant inhibition of growth, while Fe resulted in increased rate of oxalic acid decomposition (111a).

Streptomyces griseus. In surface culture Mn can be substituted for Fe on a medium containing salts-beef extract and glucose without affecting streptomycin production (3). Addition of Zn gave rise to rapid pellicle formation but inhibited streptomycin production, while Fe had a reverse effect (167). Traces of Zn, Cu, Fe and Mn were found necessary by Thornberry (156) for optimum production of streptomycin in a synthetic medium.

Saunders and Sylvester (126) report that traces of Cu, Mn, Fe and Zn were necessary ingredients of synthetic media to obtain maximum streptomycin yields when the organism was grown in submerged culture. Bennett (8) observed that much of the stimulation of streptomycin production due to cornsteep liquor could be obtained by inclusion in the medium of a proper concentration of cornsteep liquor ash, or potassium salts, such as potassium phosphate or chloride. Thornberry's recent note (157) confirms this, in that he found that ash from a number of natural materials, including peanut meal, sunflower meal, distiller's solubles and cornsteep water, increased streptomycin production when added to a medium containing glucose, peptone and NaCl.

Streptomyces scabies. One race of six tested grew on media containing 100 ppm of $HgCl_2$ (155).

Streptomyces sp. A culture resembling *S. erythreus* and *S. albosporus* required Fe for the production of actinorubin. The presence of excess Fe resulted in acid production (66).

Trichophyton rubrum and *T. gypseum.* Nickerson (101, 102) has observed that the addition of $ZnCl_2$, $Zn(NO_3)_2$, $CdSO_4$, $AgNO_3$ or $HgCl_2$ to the medium reduced the oxygen uptake of cell suspension of these organisms. The inhibition effect caused by the Zn salts could not be reversed by the addition of $CaCl_2$, $Na_2S_2O_3$ or $MgSO_4$. Addition of $CdCl_2$ increased the oxygen uptake.

DISCUSSION

While a number of advances have been made in the recognition of the effects of metallic ions on the physiology of fungi, as evidenced by the scope of the investigations mentioned, there are a number of rather basic questions still to be studied in detail. There have been numerous papers indicating that a certain effect is traceable to the presence of certain unidentified inorganic constituents (ash of a natural material—usually one of the medium constituents) (8, 9, 25, 31, 32, 33, 34, 46, 58, 68, 71, 72, 75, 108, 121, 135, 157, 159, *etc.*). Whether these effects can be attributed to a certain ion or a mixture of ions, or whether these effects are the results of imbalance of ions, or the "complexing" of an ion, is often not settled. Very often the effect may be reproduced by the addition of a single known ion or a mixture of ions, and this is taken to be proof that the effect of the ash (or similar material) was due to the presence of this ion mixture. From the data at hand it appears that the effects of one ion counteracting the effects of another ion are often overlooked in this problem, and it seems probable that this phenomenon should b considered in explaining some of the effects of the additions of such materials as ash or residues to certain media.

Thus far the problem of essentiality of any given ion for growth or a certain metabolic activity has only been touched upon. Apparently no reports are available indicating that a certain ion is required to obtain a minimum growth, a condition encountered in the study of the growth-factor requirements of fungi where specificity is very marked (128). Leonian and Lilly (81) state "The food constituents which are least affected by the specific requirements of these test organisms are the mineral elements", and this has apparently been the feeling of many other investigators who have routinely added a standard mixture of ions to various media when studying the growth-factor requirements of various organisms, or the effects of various chemical substances or physical factors on the growth or metabolism of fungi. This may often lead to erroneous conclusions, since many of these organic substances combine with metallic ions and thus affect the physiology of the test organism by making these substances unavailable to the organism. It has also been shown many times (46, 48, 71, 79, 109, 110, 145) that the addition of any one of a number of elements will result in increased growth or metabolic activity on a medium which supports only minimal growth or metabolic activity. The substitution of one element for another is especially noticeable if growth is taken at the end-point, although, as Foster (42) points out, this substitution may result in a varying composition of cellular material as well as a shunting of the carbohydrate metabolism of the mold. Perhaps this also occurs when analogues of various growth-factors are substituted; apparently, no observations have been made. The effect of these substitutions is also puzzling, for approximately the same results are apparently obtained with several substitutions (90, 106). Does this indicate that the enzymatic systems involved may be catalyzed by several different ions, or that several alternative mechanisms exist, or is this confirmation of Hutner's (53) suggestion that the contaminants in the added ions are responsible for the apparent effects. It might be noted here that very often poisonous substances, such as those containing mercury (119, 123, 134), act as growth promoters, but perhaps this is a manifestation of the Shultz-Arnst effect.

Other interesting developments are the reports concerning the effects of metallic ions on spore formation, and subsequent fermentations by these spores (111, 132). An explanation for this phenomenon is that certain metallic ions favor the formation of a certain type of spore, or perhaps the composition of the spore varies with the metallic ion content of the sporulation medium. This may account in part for the apparent variation in cultures observed by Chrzaszcz and Zakomorny (29). This observation is in line with the effect of metallic ions on the composition of the cellular material (112, 131).

A consideration of the literature cited in this review suggests that several rather basic criticisms may be applied to many of the investigations reported, and in turn these criticisms limit any generalizations that may be drawn from the observations at hand. Since many of the reports deal with *A. niger,* the work on this organism may be used as an illustration. The reports indicate that a large number of different cultures have been used which in turn have been obtained from various sources and consequently have different histories. Usually these cultures have been grown on different media and under different cultural conditions in each laboratory. Very rarely is the trace element content of the medium stated or mentioned, and a comparison (111) has indicated that different strains of the organism react differently under the same cultural conditions when grown on the same medium. It has been reported that such matters as temperature of incubation will cause radical changes in growth (13) as well as metabolism of various strains (111). Until some effort is made to standardize techniques, as growing inoculum, sporulation, methods of preparing stock cultures, preparation of media, *etc.,* it is inadvisable to draw any general conclusions regarding the metabolism of carbohydrates by fungi, or the effects of metallic ions on the metabolism, as indicated by the few comparisons that have been made between cultures grown under different laboratory conditions (13, 107, 111, 132).

The above discussion has mentioned only a few of the prominent problems that exist on this general topic. During the nine years covered by this review, a number of subjects have generally been recognized in the various laboratories studying physiology of fungi, including metallic ion antagonism, lack of specificity of metallic ions for certain responses, and the indirect effects of metallic ions on the fermentation by their effects on sporulation. However, these topics are not new and have been mentioned in the literature many years ago. Perhaps the investigations of the next decade will resolve some of these old problems as well as open others.

BIBLIOGRAPHY

1. ABRAHAM, E. P., *et al.* Further observations on penicillin. Lancet. **241**: 177–188. 1941.
2. ACOCK, A. M. An examination of Mulder's rapid biological method for estimating the amount of copper in soils. Jour. Australian Coun. Sci. & Ind. Res. **14**: 288–300. 1941.
3. AINSWORTH, G. C., *et al.* A method for the large scale production of streptomycin by surface culture. Jour. Gen. Microbiol. **1**: 335–343. 1947.
4. ALBERT, A, and GLEDHILL, W. S. The choice of a chelating agent for inactivating trace metals. I. Survey of commercially available chelating agents. Biochem. Jour. **41**: 529–533. 1947.
5. ———— and MCGRATH, D. The choice of a chelating agent for inactivating trace metals. II. Derivatives of oxine (8-hydroxyquinoline). Biochem. Jour. **41**: 534–545. 1947.
6. ———— *et al.* The influence of chemical constitution on antibacterial activity. III. A study of 8-hydroxyquinoline (oxine) and related compounds. Brit. Jour. Exp. Path. **28**: 69–87. 1947.
7. BAILEY, J. H., and CAVALLITO, C. J. Production of citrinin. Jour. Bact. **45**: 30–31. 1943.
8. BENNETT, R. E. Nutrition of *Streptomyces griseus* in relation to streptomycin titer. Jour. Bact. **53**: 254. 1947.
9. BERNHAUER, K. *et al.* X. [Influence of the kind of sugar on citric acid formation]. [German]. Biochem Zeits **307**: 298–308. 1941.
10. ————*et al.* [Formation of acid from sugar by *A. niger*. XI. Factors determining the accumulation of citric acid]. [German]. Biochem. Zeits. **309**: 151–178. 1941.
11. BERTRAND, D. [Importance of the trace-element vanadium for *Aspergillus niger*]. [French]. Compt. Rend. Acad. Sci. (Paris) **213**: 254–257. 1941.
12. ————. [Vanadium as a growth factor for *Aspergillus niger*]. [French]. Bull. Soc. Chim. Biol. **23**: 467–471. 1941.
13. ————. [V, an oligosynergic element for *Aspergillus niger*]. [French]. Ann. Inst. Pasteur **68**: 226–244. 1942.
14. Brit. Intel. Off. Sub-com., Final Rep. 220. Production of citric acid at the factory of Joh. A. Benckiser, Lardenburg, near Heidelberg. British Intelligence Sub-Committee, 32 Bryanston Sq., London, W.C.1. 1946.
15. ———— Final Rep. 489. Chemische Fabrik Joh. A. Benckiser G.M.B.H. Manufacture of calcium citrate. British Intelligence Sub-Committee, 32 Bryanston Sq., London, W.C.1. 1946.
16. BIRD, M. L., and CHALLENGER, F. Formation of organometalloidal and similar compounds by microorganisms. VII. Dimethyl telluride. Jour. Chem. Soc. **1939**: 163-168.
17. ———— and ————. Biological methylation. IX. The action

of *Scopulariopsis brevicaulis* and certain penicillia on salts of aliphatic selenic and selenonic acids. Jour. Chem. Soc. **1942:** 574–577.

18. Birkinshaw, J. H. *et al.* Patulin in the common cold. II. Biochemistry and chemistry. Lancet. **243:** 625–630. 1943.

19. ———— *et al.* Studies in the biochemistry of microorganisms. LXXII. Gentisyl alcohol (2:5 dihydroxybenzyl alcohol), a metabolic product of *P. patulum* Bainier. Biochem. Jour. **37:** 726–728. 1943.

19*a*. Blank, L. M. Response of *Phymatotrichum omnivorum* to certain trace elements. Jour. Agr. Res. **62:** 129–160. 1941.

20. Boehm, R. M. *et al.* Clarified sugar solutions for fermentation. U. S. Patent 2,421,985. 1947.

21. Bojanovsky, R. [The need for iron by the cellulose-splitting fungi and their relationship to the conditions under which ferric ions are chemically adsorbed]. [German]. Zentr. Bakt. Parasitenk. II. Abt. **99:** 48–55. 1938.

22. ————. [Pure cultures of fungi obtained by the selective action of ions]. [German]. Zentr. Bakt. Parasitenk. II. Abt. **99:** 55–60. 1938.

23. Bonner, D., *et al.* The genetic control of biochemical reactions in *Neurospora:* A mutant strain requiring isoleucine and valine. Arch. Biochem **8:** 71–91. 1943.

24. Brack, A. [Antibacterial compounds. I. The isolation of gentisyl alcohol in addtion to patulin from the filtrate of a penicillium culture. Some derivatives of gentisyl alcohol]. [German]. Helv. Chim. Acta **30:** 1–8. 1947.

25. Brian, P. W. Production of gliotoxin by *Penicillium terlikowskii* Zal. Trans. Brit. Mycol. Soc. **29:** 211–218. 1946.

26. Burk, D., *et al.* Cobalt inhibition of tissue respiration, glycolysis and growth. Fed. Proc. **5:** 126–127. 1946.

27. Carpenter, C. C., and Friedlander, E. W. Occurrence of vitamins in fungi. Science **95:** 625. 1942.

28. ———— and Strong, F. M. Determination of pyridoxin pseudopyridoxin. Arch. Biochem. **3:** 375–388. 1944.

29. Chrzaszcz, T., and Zakomorny, M. [The degeneration of biochemical properties of strongly acidifying *Aspergillus niger* cultures]. [Polish]. Przemsl. Chem. **22:** 296–302. 1938.

30. Clayton, G. E. *et al.* Preparation of penicillin. Improved method of isolation. Biochem. Jour. **38:** 452–458. 1944.

31. Cook, R. P., and Brown, M. B. Some constituents of aqueous extracts of ground dried peas. Biochem. Jour. **39:** 24. 1945.

32. ———— *et al.* The production of penicillin using fractions obtained from aqueous extracts of pea (*Piseum sativum*). Biochem. Jour. **39:** 314–317. 1945.

33. ———— *et al.* Factors in aqueous extracts of peas responsible for penicillin production. Biochem. Jour. **39:** 23. 1945.

34. Corum, C. J. Carbohydrate nutrition of *Rhizopus suinus.* Bot. Gaz. **103:** 740–750. 1942.

35. Coulthard, C. E. *et al.* Notatin: an antibacterial glucose-aerodehydrogenase from *Penicillium notatum* Westling and *Penicillium resticulosum* sp. nov. Biochem. Jour. **39:** 24–36. 1945.

36. Curtin, T., *et al.* Production of phoenicine on synthetic media. I. *Penicillium phoenic eum* van Beyma. II. *Penicillium rubrum* Grasberger-Stoll. Biochem. Jour. **34:** 1605–1610. 1940.

37, Dillon-Weston, W. A. R., and Taylor, R. E. Development of *Penicillium* on the cut surfaces of certain vegetables. Nature **151:** 54–55. 1943.

38. ——— and ———. Development of mold on the cut surface of potato tubers. Jour. Agr. Sci. **34:** 93–96. 1944.

39. Erb, N. M., and Hilderbandt, F. M. Mold as an adjunct to malt in grain fermentation. Ind. Eng. & Chem. **38:** 791–794. 1946.

39*a*. Ezekiel, W. N. Synthetic culture media for the root-rot fungus *Phymatotrichum omnivorum*. Phytopath. **35:** 159–161. 1945.

40. Fischer, J. [The effect of heavy metals on *Aspergillus niger*. I. Starch decomposition]. [German]. Planta **32:** 395–413. 1942.

41. Foster, J. W. Heavy metal nutrition of fungi. Bot. Rev. **5:** 207–239. 1939.

42. ———. Some introspections on mold metabolism. Bact. Rev. **11:** 167–188. 1947.

43. ———, *et al.* Microbiological aspects of penicillin. V. Conidiospore formation in submerged cultures of *Penicillium notatum*. Jour. Bact. **50:** 365–368. 1945.

44. ———, and Waksman, S. A. Production of fumaric acid by molds belonging to the genus *Rhizopus*. Jour. Am. Chem. Soc. **16:** 127–135. 1939.

45. ———, *et al.* Microbiological aspects of penicillin. III. Production of penicillin in surface cultures of *Penicillium notatum*. Jour. Bact. **46:** 421–433. 1943. (Abst.: Foster, J. W. Microbiological aspects of penicillin formation. Jour. Bact. **45:** 65). 1943.

46. ———, *et al.* Microbiological aspects of penicillin. IV. Production of penicillin in submerged cultures of *Penicillium notatum*. Jour. Bact. **51:** 465–478. 1946.

47. Fries, N. [On the importance of growth-factors for the growth of fungi]. [German]. Sym. Bot. Upsaliensis **3:** 1–188. 1938.

48. Gastrock, E. A., *et al.* Gluconic acid production on pilot-plant scale; effect of variables on production by submerged mold growth. Ind. Eng. & Chem. **30:** 782–789. 1938.

49. Gerhardt, P., *et al.* Citric acid fermentation of beet molasses. Jour. Bact. **52:** 555–564. 1946.

50. Gilbert, W. J. and Hickey, R. J. Production of conidia in submerged cultures of *Penicillium notatum*. Jour. Bact. **51:** 731–733. 1946.

51. Hubert, B. [Copper tolerance of *Penicillium waksmanii* Zaleski. Biol. Jaarb. Natuurw. Genootsch. Dodonaea **5:** 326–329]. [Dutch]. (Biol. Abst. **14:** 7573.)

52. Hutner, S. H. Unidentified trace element requirements of photosynthetic purple bacteria. Jour. Bact. **51:** 575–576. 1946.

53. ———. Organic growth essentials of the aerobic nonsulfur photosynthetic bacteria. Jour. Bact. **52:** 213–221. 1946.

54. Iuracec, A. [Influence of iron on the acidity variations of the nutrient solution of*Aspergillus niger*.] [Roumanian]. Bull. Sect. Sci. Acad. Roumaine **19:** 209–212. (Chem. Abst. **34:** 3301). 1937–8.

55. Jarvis, F .G. Synthetic media for the production of penicillin. M. Sc. Thesis, Univ. Wis. 1946.

56 ———, and Johnson, M. J. The role of the constituents of synthetic media for penicillin production by *P. chrysogenum* Q-176 in shake flasks. Abst. Am. Chem. Soc. Chicago meeting, Sept. 9–13, p 12B–13B. 1946.

57. ———, and ———. The role of the constituents of synthetic media for penicillin production. Jour. Am. Chem. Soc. **69:** 3010–3011. 1947.

58. Javillier, M. M. [Copper and *Aspergillus niger*]. [French]. Ann. Ferment. (Paris) **5:** 371–381. 1939.

59. Joslyn, M. A. The mineral metabolism of yeast. Wallerstein Lab. Comm. **4:** 49–64. 1941.

60. Karow, E. O. Production of citric acid in submerged culture. Ph.D. Thesis, Rutgers Univ. 1942.

61. ———. Factors affecting the production of citric acid in submerged cultures. Jour. Bact. **54**: 41. 1947.

62. ———, and Waksman, S. A. Production of citric acid in submerged culture. Ind. Eng. & Chem. **39**: 821–5. 1947.

-63. Kauffmann-Cosla, O., and Brull, R. [Action of zinc on cellular resorption]. [German]. Arch. Int. Pharmacodynamie **63**: 326–35. 1939.

-64 ——— *et al.* [Biological action of zinc]. [German]. Bull. Soc. Chim. Biol. **21**: 422. 1939. (Chem. Abst. **33**: 5432).

65 ——— *et al.* [Effect of ions on the germination and development of the spores of *A. niger*]. [French]. Rev. Gen. Bot. **52**: 97–111. 1940.

66 Kelner, A., and Morton, H. E. Two antibiotics (lavendulin and actinorubin) produced by actinomycetes. I. Isolation and characteristics of the organisms. Jour. Bact. **53**: 695–704. 1947.

67. Kind, A. [Acid production of fungi and its influence on timbers impregnated with $CuSO_4$]. [French]. Bull. Schwiez. Elektrotech. Ver. **35**: 174–261. 1945. (Rev. Appl. Myc. **25**: 284).

68. Kita, D A. Factors affecting the production of citric acid from molasses by*Aspergillus niger*. B. S. Thesis, Univ. Wis. 1944.

-69. Kitavin, G. S. [Action of mercury salts on the formation of B_2 in *A. niger*]. [Russian]. Biokhimiya **4**: 283–94. 1939.

70. ———. [Crystalline riboflavin obtained through the action of mercuric salts on *A. niger*]. [Russian]. Compt. Rend. Acad. Sci. U.S.S.R. **28**: 517–18. 1940.

71. Knight, S. G. The effect of certain inorganic constituents on penicillin production and mold metabolism in shake flask fermentations. Ph.D. Thesis, Univ. Wis. 1946.

72. ———, and Frazier, W. C. The effect of cornsteep liquor ash on penicillin production. Science **102**: 617–618. 1945.

73. Knobloch, H., and Sellmann, R. [The formation of flavintype pigments in liquid cultures of *Aspergillus niger*]. [German]. Zentr. Bakt. Parasitenk. II Abt. **103**: 277–80. 1941.

74. ——— and Miksch, J. N. [Formation of acid from sugar by *A. niger*. XIII. Influence of H_2SeO_3 on the citric acid production by *A. niger*]. [German]. Biochem. Zeits. **309**: 90–9. 1941.

75. ——— and Sellmann, R. [Influence of trace elements in water on acid production by strains of *A. Niger*]. [German]. Biochem. Zeits. **309**: 145–150. 1941.

76. Koffler, H., *et al.* The effect of certain chemicals on penicillin production and mold metabolism in shake-flask fermentation. Jour. Bact. **50**: 549–59. 1945.

77. ———, *et al.* Metabolic changes in submerged penicillin fermentations on synthetic media. Jour. Bact. **51**: 385–392. 1946.

78. ———, *et al.* The effect of certain mineral elements on the production of penicillin in shake flasks. Jour. Bact. **53**: 115–123. 1947.

79. Leonian, L. H., and Lilly, V. G. Studies on the nutrition of fungi. IV. Factors influencing the growth of some thiamine fungi. Am. Jour. Bot. **27**: 18–26. 1940.

80. ———, and ———. Studies on the nutrition of fungi. V. Factors affecting zygospore formation in *Phycomyces blakesleeanus*. Am. Jour. Bot. **27**: 670–5. 1940.

81. ———, and ———. The comparative value of different test organisms in the microbiological assay of the B vitamins. W. Va. Agr. Exp. Sta. Bull. 319. 1945.

82. Lochhead, A. G., *et al.* Production of claviformin by soil penicillia. Canad. Jour. Res. (E) 24: 1–9. 1946.

83. Lockwood, L. B. *Rhizopus oryzae:* spore germination, early growth and fermentation. Rep. Proc. III Congr. Microbiol. 1939, p. 229. 1940.

84. ————, and Reeves, M. D. Some factors affecting the production of itaconic acid by *Aspergillus terreus.* Arch. Biochem. 6: 455–469. 1945.

85. ————, and Nelson, G. E. N. Some factors affecting the production of itaconic acid by *Aspergillus terreus* in agitated cultures. Arch. Biochem. 10: 365–374. 1946.

86. Lohrmann, W. [The antagonistic action of magnesium against boron and mercury for some fungi]. [German]. Arch. Microbiol. 11: 329–367. 1940.

87. Lorenz, W. Improvements relating to the production of organic acids by fermentation. Brit. Patent 517,793. 1940.

88. Menzel, A. E. O. *et al.* The isolation of gliotoxin and fumigacin from culture filtrates of *Aspergillus fumigatus.* Jour. Biol. Chem. 152: 419–429. 1944.

89. Mezzadroli, G. [Procedure for the production of citric acid by fermentation of carbohydrates]. [French]. French Patent 833,631. 1938.

90. Moyer, A. J. and Coghill, R. D. The laboratory-scale production of itaconic acid by *Aspergillus terreus.* Arch. Biochem. 7: 167–183. 1945.

91. ————, and ————. Penicillin. VIII. Production of penicillin in surface cultures. Jour. Bact. 51: 57–78. 1946.

92. ————, and ————. Penicillin. IX. The laboratory scale production of penicillin in submerged cultures by *Penicillium notatum* Westling (NRRL 832) Jour. Bact. 51: 79–93. 1946.

93. Mulder, E. G. [The importance of copper for the growth of microorganisms and a microbiological method of estimation of soil copper available to plants]. [German]. Arch. Mikrobiol. 10: 72–96. 1939.

94. Mull, R. P., and Nord, F. F. On the mechanism of enzyme action, Part 23. Structure and action of rubrofusarin from *Fusarium graminearum* Schwabe (Fgra) (*Gibberella saubinettii*) Arch. Biochem. 4: 419–433. 1944.

95. Nagasako, S. [Applicability of biological reactions of *A. niger* for microdetermination of heavy metals in blood]. [Japanese]. Japan Jour. Med. Sci. VII. Social Med. Hyg. 3: (1–2). Proc. Japan Soc. Forensic Med. 23: 20. 1939. (Chem. Abst. 35: 5140).

96. Nakazawa, R., and Sino, M. [Effect of irradiation on fermentative organisms. I. Morphological and biochemical characteristics of races of *A. niger* produced by radium treatment]. [Japanese]. Jour. Agr. Chem. Soc. (Japan) 14: 895–910. 1938.

97. ————, and ————. [The action of Ra and x-rays on microorganisms. II. Production of acids by Ra irradiated *A. niger.*] [Japanese]. Jour. Agr. Chem. Soc. (Japan) 15: 547-52. 1939.

98. ————, and ————. Effect of radioactive substances on fermenting microorganisms. Rep. Proc. III Int. Cong. Microbiol. 1939. p. 753–754. 1940.

99. Negroni, P., and Fischer, I. [Inhibiting action of *Penicillium notatum.* I. Influence of nutritive source of C]. [Spanish]. Rev. Inst. Bact. "Carlos G. Malbran" 12: 369–373. 1944.

100. Neuberg, C. The biochemistry of yeast. Ann. Rev. Biochem. 15: 435–474. 1946.

101. Nickerson, W. J. Inhibition of fungus respiration: a metabolic bio-assay method. Science 103: 484–486. 1946.

102. ————, and Chadwick, J. B. On the respiration of dermatophytes. Arch. Biochem. 10: 81–100. 1946.

103. Nielsen, N. The growth substance demand of different fungi. Rep. Proc. III Int. Cong. Microbiol. 1939. p. 204. 1940.

104. Niklas, H., and Toursel, O. [The determination of trace elements by means of *A. niger*]. [German]. Bodenkunde u. Pflanzenernahr 23: 357–360. 1941. (Chem. Abst. 36: 956).

105. Perlman, D. Factors affecting the production of citric acid by *Aspergillus niger.* M.Sc. Thesis, Univ. Wis. 1943.

106. ————. Some effects of metallic ions on the metabolism of *Aerobacter aerogenes.* Jour. Bact. 49: 167–175. 1945.

107. ————. Penicillin production in submerged culture. Ph.D. Thesis, Univ. Wis. 1945.

108. ————, *et al.* Production of citric acid from cane molasses. Arch. Biochem. 11: 123–129. 1946.

109. ————, *et al.* Effect of metallic ions on the production of citric acid by *Aspergillus niger.* Arch. Biochem. 11: 131–143. 1946.

110. ————. On the nutrition of *Memnoniella echinata* and *Stachybotrys atra.* Am. Jour. Bot. 35: 36–41. 1948.

111. ————. Citric acid production by various strains of *Aspergillus niger.* Abst. Comm., IV Int. Cong. Microbiol. p. 201–202. 1947.

111*a*. ————. On the nutrition of *Sclerotium delphinii.* Am. Jour. Bot. 35: 360–363 (1948).

112. Pontovich, V. E. [Influence of potassium on the synthesis of proteins in fungi.] [Russian]. Bull. Acad. Sci. U.S.R.R. Biol. p. 191–203. 1942. (Chem. Abst. 37: 3122).

113. ————. [Light and potassium in the development of molds]. [Russian]. Bull. Acad. Sci. U.S.S.R. Biol: 612–21. 1945. (Chem. Abst. 40: 5095).

114. Porges, N., *et al.* Gluconic acid production. Repeated and re-use of submerged *Aspergillus niger* by filtration. Ind. Eng. & Chem. 33: 1065–1067. 1941.

115. Pratt, R., and Dufrenoy, J. (Letter to editor) Science 105: 574. 1947.

116. Ratajak, E. J. and Owens, H. S. Optimal condition for the hydrolysis of arabogalactan by *Aspergillus niger.* Bot. Gaz. 104: 329–337. 1942.

117. Rawlings, F. N. Purification of sugar solutions. U.S. Patent 2,391,843. 1945.

118. ———— and Shafor, R. W. Ionic exchangers: their application in cane and beet sugar juice purification. Sugar 37: 1–6. 1942.

119. Rich, S., and Horsfall, J. G. Metal reagents as antisporulants. Phytopath. 38: 22. 1948.

120. Rippel, A., and Lohrmann, W. [Effect of nutrients and poisons]. [German]. Nachr. Ges. Wiss. Gottingen, Math.-Phys. Klasse. Fachgruppe VI (N.F.) 3: 239–253. 1940. (Chem. Abst. 35: 5931).

121. Robbins, W. J. Growth substances in agar. Am. Jour. Bot. 26: 772–778. 1939.

122. Roberts, E. C., *et al.* Penicillin B, an antibacterial substance from *Penicillium notatum.* Jour. Biol. Chem. 147: 47–57. 1943.

123. Robertson, M. E. Apparent stimulative effect on mold growth of a mercurial preparation. Nature 151: 365. 1943.

123*a*. Rogers, C. H. Growth of *Phymatotrichum omnivorum* in solutions with varying amounts of certain mineral elements. Am. Jour. Bot. 25: 621–624. 1938.

124. Rubbo, S. D. The mode of action of 8-hydroxyquinoline—a biological approach. Abst. Comm., IV Int. Cong. Microbiol. p. 31–32. 1947.

125. Ryan, F. J., *et al.* The tube method of measuring the growth rate of *Neurospora.* Am. Jour. Bot. **30**: 784–799. 1943.

126. Saunders, A. P., and Sylvester, J. C. Synthetic media for the production of streptomycin. Abst. Am. Chem. Soc. Meeting, Chicago, Ill., Sept. 15–19, p. 9A–10A. 1947.

127. Schopfer, W. H. [Biosynthesis of biotin by *Phycomyces blakesleeanus.* New growth-factor for yeast]. [French]. Zeits. Vitaminf. **14**: 42–70. 1943.

128. ————. Plants and vitamins. 1943.

129. ————. [Comments regarding Utiger's article: Vitamin B_1 studies of the *Phycomyces* growth test. Critical examination of its application]. [German]. Zeits. Vitaminf. **18**: 66–70. 1946.

130. ————, and Utiger, H. [Sea water as a source of metallic catalysts for the culture of a microorganism]. [French]. Compt. Rend. Soc. Phys, et. Hist. Nat. (Geneva) **58**: 135–37. 1941.

131. Schulz, G. [The influence of a few heavy metals (Zn,Cd,Mn,Fe) on the chemical composition of *Aspergillus niger.*]. [German]. Planta **27**: 196–218. 1937.

132. Shu, P., and Johnson, M. J. Citric acid production by submerged fermentation. Abst. Am. Chem. Soc. Meeting, Atlantic City, N. J., April 14–18, p 11A–12A. 1947.

133. ————, and ————. Effect of the composition of the sporulation medium on citric acid production by *Aspergillus niger* in submerged culture. Jour. Bact. **54**: 161–168. 1947.

133*a*. ————, and ————. Citric acid production by submerged fermentation with *Aspergillus niger.* Ind. Eng. & Chem. **40**: 1202–1205 (1948).

133*b*. ————, and ————. The interdependence of medium constituents in citric acid production by submerged fermentation. Jour. Bact. **56**: 577–585 (1948).

134. Singh, J. Effect of Hg on microorganisms. Cur. Sci. **13**: 236. 1944.

135. Sjolander, J. R. Factors affecting the production of citric acid in submerged mold cultures. B.A. Thesis, Univ. Wis. 1945.

136. Smit, Jan, and Mulder, E. G. [Biological estimation of copper and magnesium in soils and plants]. [French]. Rec. Trav. Chim. Pays-Bas **59**: 623. 1940.

137. Starkey, R. L. and Waksman, S. A. Fungi tolerant to extreme acidity and high concentration of copper sulfate. Jour. Bact. **45**: 509–519. 1943.

138. Stefaniak, J. J., *et al.* Pilot plant equipment for submerged production of penicillin. Ind. Eng. & Chem. **38**: 666–671. 1946.

139. Steinberg, R. A. Effects of N compounds and trace elements on growth of *A. niger.* Jour. Agr. Res. **59**: 731–748. 1939.

140. ————. Relation of carbon nutrition to trace element and accessory requirements of *A. niger.* Jour. Agr. Res. **59**: 749–763. 1939.

141. ————. Action of some organic compounds on yield, sporulation, and starch formation of *Aspergillus niger.* Jour. Agr. Res. **60**: 765–773. 1940.

142. ————. Relation of accessory substance and amino acid requirements to the C nutrition of *A. niger.* Rep. Proc. III Int. Cong. Microbiol. 1939. p 486. 1940.

143. ————. Sulfur and trace element nutrition of *A. niger.* Jour. Agr. Res. **63**: 109–127. 1941.

144. ———. Effect of trace elements on growth of *A. niger* with amino acids. Jour. Agr. Res. **64**: 455–475. 1942.

145. ———. The process of amino acid formation from sugars in *A. niger*. Jour. Agr. Res. **64**: 615–633. 1942.

146. ———. Influence of carbon dioxide on response of *Aspergillus niger* to trace elements. Pl. Phys. **17**: 129–132. 1942.

147. ———. Use of microorganisms to determine essentiality of minor elements. Soil Sci. **60**: 185–189. 1945.

148. ———. Dibasal (minimal salt, maximum yield) solution for *Aspergillus niger*; acidity and magnesium optimum. Pl. Phys. **20**: 600–608. 1945.

149. ———. Specificity of potassium and magnesium for growth of *Aspergillus niger*. Am. Jour. Bot. **33**: 210–214. 1946.

150. Stiles, W. Trace elements in plants and animals. 1946.

151. Stokes, J. L., *et al.* A *Neurospora* assay for pyridoxine. Jour. Biol. Chem. **150**: 17–24. 1943.

152. ———, *et al.* Synthesis of pyridoxin by a "pyridoxinless" x-ray mutant of *Neurospora sitophila*. Arch. Biochem. **2**: 235–245. 1943.

153. Stout, P. R., and Arnon, D. I. Experimental methods for the study of the role of copper, manganese, and zinc, in the nutrition of higher plants. Am. Jour. Bot. **26**: 144–149. 1939.

154. Texera, D. A. Production of antibiotic substances by *Fusaria*. Phytopath. **38**: 70–81. 1948.

155. Thomas, W. D. Growth and variation of six physiologic races of *Actinomyces scabies* on different media. Phytopath. **37**: 319–331. 1947.

156. Thornberry, H. H. Nutrient requirements of an antibiotic soil fungus, *S. griseus*. Phytopath. **36**: 412. 1946.

157. ———. The role of minerals in production of streptomycin by *Streptomyces griseus*. Phytopath. **38**: 26. 1948.

158. Timmonin, M. I., and Rouatt, J. W. Production of citrinin by *Aspergillus* sp. of the candidus group. Canad. Jour. Pub. Health **35**: 80–88. 1944.

159. Utiger, H. and Schopfer, W. H. [Additional information on the symbiosis between *Rhodotorula rubra* and *Mucor ramannianus*. The role of metallic catalysts]. [French]. Compt. Rend. Soc. Phys. et Hist. Nat. (Geneva) **58**: 284–88. 1941. (Also mentioned in Schopfer (128) p. 249).

160. ———. [Studies of the vitamin B_1 content of vegetables by means of the *Phycomyces* growth test]. [German]. Zeits. Vitaminf. **18**: 35–65. 1946.

161. Waksman, S. A., and Foster, J. W. The mechanism of lactic acid production by *Rhizopus*. Jour. Bact. **35**: 70–71. 1938.

162. ———, *et al.* Two antagonistic fungi, *Aspergillus fumigatus* and *Aspergillus clavatus*, and their antibiotic substances. Jour. Bact. **45**: 233–248. 1943.

163. ———, and Karow, E. O. Citric acid production by fermentation. U. S. Patent 2,394,031. 1946.

164. Waring, W. S., and Werkman, C. H. Growth of bacteria in an iron-free medium. Arch. Biochem. **1**: 303–310. 1943.

165. Weitz, F. W. Juice purification by ion exchange. Sugar **38**: 1–6. 1943.

166. Wickerham, L. J., *et al.* The production of riboflavin by *Ashbya gossypii*. Arch. Biochem. **9**: 95–98. 1946.

167. Woodruff, H. B. Production of streptomycin in stationary culture on liquid and solid substrate. Jour. Bact. **54**: 42. 1947.

168. ———. The bacteriological spectra and microbiological synthesis of the natural penicillins. Abst. Comm., IV Int. Cong. Microbiol. p 10–11. 1947.
169. WOODWARD, J. C., *et al.* Treatment of corbohydrates for fermentation. Canad. Patent 422,142. 1944.
170. WORLEY, C. B. Synthetically produced substance B. Bot. Gaz. **103**: 391–396. 1941.
171. ———. Agencies affecting the production of substance B by *Rhizopus suinus.* Pl. Phys. **16**: 461–480. 1941.
172. WYLLIE, J. Method for the rapid production of citrinin. Canad. Jour. Pub. Health **36**: 477–483. 1945.
173. YOSHIMURA, F. [The action of some heavy metals upon the production of catalase in aspergilli]. [Japanese]. Bot. Mag. (Tokyo) **53**: 125–128. 1939. (Biol. Abst. **13**: 15779).
174. ZALDEVAR, J. P. The influence of $FeCl_3$ and of $ZnSO_4$ on the production of citric acid from cane sugar solutions by fermentation with *Aspergillus niger.* Phillipine Agr. **29**: 738–752. 1941.
175. ZENTMEYER, G. A. Inhibition of metal catalysis as a fungistatic mechanism Science **100**: 294–295. 1944.

THE BOTANICAL REVIEW

VOL. XV APRIL, 1949 No. 4

RECENT DEVELOPMENTS IN SEED TECHNOLOGY

R. H. PORTER
American International Association, Brazil

INTRODUCTION 221
IMPORTANT DEVELOPMENTS 223
METHODS OF SEED IDENTIFICATION 224
  Physical Methods 224
  Chemical Methods 227
  Morphological Methods 228
  Pathological Methods 232
UNIFORM METHOD FOR DETERMINING PURITY OF SMALL-SEEDED GRASSES 234
A PRACTICAL CONCEPT OF PURE SEED 237
EVALUATION OF WEED SEEDS IN CROP SEED 242
MORE UNIFORM EVALUATION OF SEED GERMINATION TESTS 245
ADVANCES IN OUR KNOWLEDGE OF IMPERMEABLE SEEDS ... 252
ADVANCES IN OUR KNOWLEDGE OF DORMANT SEEDS 262
DEVELOPMENT OF METHODS THAT PROLONG SEED VIABILITY 272
  Seeds of Field Crops and Grasses 273
  Seeds of Horticultural and Floral Plants 275
  Seeds of Trees and Shrubs 278

INTRODUCTION

A recent historical sketch by Brown (52) pointed out that seed testing as a service to those who wished to know something of seed quality was first undertaken in Denmark and Germany in 1869, although as early as 1816 Switzerland passed a seed law which required that at least two men in a county, where clover seed was sold, were to serve as inspectors of seed offered for sale. Seed testing developed rapidly in Europe after 1869, and many small testing and control stations were established which later were consolidated into one or more well equipped central stations in each country.

The earliest development in this field on the American continent was in Connecticut in 1821 when the first law regulating the sale of seed in the United States was passed. Relatively little progress was made, however, until 1876 when Dr. E. H. Jenkins, who had visited Nobbe's laboratory in Germany, discussed in a report of the Connecticut Station the value of seed testing to the United States.

In the early work with seeds the major effort was to determine the identity of weed seeds and their importance when present in crop seeds. It was natural that botanists who were interested in this aspect of applied botany should give attention to seed identification including both weed and crop seed. Botanists from a considerable number of States published bulletins which were well illustrated with pictures and drawings of seed. Determination of seed purity naturally followed the emphasis on seed identification. But of almost equal importance was the measurement of seed viability which was early recognized as an important factor in seed quality.

One of the earliest problems that confronted those who were interested in seed testing was how to make analyses for purity and tests for germination. The American Seed Trade Association received a request in 1889 from the North Carolina Agricultural Experiment Station to assist in preparing a standard of seed quality, but no progress in that direction was made at that time. A memorial signed in 1896 by a majority of the Agricultural Experiment Station Directors was presented to the Association of American Agricultural Colleges and Experiment Stations in which a request was made for the appointment of a "committee of experts in seed testing to devise and adopt a standard form of seed testing apparatus and method of procedure for use in all American Stations". In 1897 the committee which had been appointed formulated rules for seed testing that were subsequently revised and published in 1904 and 1906. The Association of Official Seed Analysts of North America was organized in 1908, and from that time until 1939 the responsibility of formulating rules for seed testing was undertaken primarily by that Association. In 1939 (400) a new Federal Seed Act was passed, and a set of rules and regulations to be used as a guide in the enforcement of that Act was drawn up, published and put into effect in February, 1940, by the Agricultural Marketing Service of the United States Department of Agriculture.

The first international meeting of seed analysts was held in 1905 in connection with the Botanical Congress in Wien, and in 1921 the International Seed Testing Association was formed. The initial purpose of this Association as well as of the American Association of Official Seed Analysts was to promote uniformity of testing methods to the end that a freer exchange of seed in commerce might be possible.

From the beginning of seed testing it became evident that the success of the practice would depend on (*a*) the development of new information about seeds and (*b*) the application of many principles and techniques of botanical science to the art of seed testing. Within the past 75 years the contributions to our knowledge of the taxonomy, physiology, morphology, dormancy, viability, impermeability, longevity and pathology of seeds by investigators in Europe, North America and other parts of the world have been extensive. All of these contributions have aided in the development of seed laboratories whose function has been the determination of seed quality in terms of purity and vitality. Both laboratory and field methods have been employed in this extensive enterprise.

It is the purpose of this paper to discuss some of the recent developments in seed technology that have advanced our general knowledge of seeds, have been of considerable value to seed analysts and have contributed to the welfare and advancement of the seed industry and of agriculture in general.

IMPORTANT DEVELOPMENTS

The more important developments that have occurred in seed technology within the past decade or more are:

1. New and improved methods of seed identification, respecting both variety and species,
2. A uniform method for the determination of pure seed in small-seeded grasses,
3. A practical concept of pure seed,
4. Evaluation of weed seeds in crop seed,
5. More uniform evaluation of seed germination tests,
6. Advances in our knowledge of the value and treatment of impermeable seeds,
7. Advances in our knowledge of dormant seeds,
8. Development of methods for prolonging seed longevity,

9. Development of rapid methods for the determination of seed viability,
10. A knowledge of the relationship of hormones and inhibitors to seed germination,
11. Recognition of the importance of seed-borne organisms in seed laboratory practice,
12. Use of seed fungicides in seed testing,
13. Development of laboratory methods for measuring seed germinability under adverse conditions,
14. Application of statistical methods to seed technology.

METHODS OF SEED IDENTIFICATION

A major objective in seed analysis from the earliest beginnings has been the identification of seeds. It was early recognized that the seeds of certain plant families possess definite characteristics as to both external and internal morphology, and an understanding of these family characteristics is of great value to the analyst. For example, the structure of seeds (fruits) in the Gramineae is so distinctive that an experienced analyst has no difficulty in recognizing a naked fruit or one covered with glumes as belonging in that family. Not all plant families have such distinctive external features as do the seeds of Gramineae, but in many cases there are features that are peculiar to a family. Within families the problem is more complicated, as between genera, species and varieties. The normal seeds of *Festuca elatior* L. and of *Lolium perenne* L. as found in commerce are strikingly similar, yet there are recognizable differences. Within genera, particularly *Lolium, Poa, Agrostis* and *Brassica,* certain species are so similar that differentiation is difficult if not impossible by seed examination alone. There are frequently varieties within a species whose seeds are so strikingly similar that no external or internal features are adequate for varietal differentiation. It is often important from the standpoint of either the farmer or the seed merchant to determine the identity of a given lot of seed; hence efforts have been made to find satisfactory methods. The methods that have been employed are physical, chemical, morphological and pathological.

Physical Methods. The most common physical method recently developed and now employed by many seed laboratories is the use of filtered, ultra-violet light as a means of distinguishing the seeds of *Lolium perenne* from those of *L. multiflorum* Lam. Gentner recorded his observations in experiments with seedlings of these two species when exposed to filtered, ultra-violet light. He discovered that the roots of seedlings of the latter when grown on filter paper produced a substance that was fluorescent in the presence of filtered ultra-violet light, whereas the roots of seedlings of the former were non-fluorescent. Furthermore, the substance produced by fluorescent seedlings was found to remain for a long time in the paper, even when dry. This discovery promised to have considerable practical importance because *L. multiflorum* is an annual and of less value economically than *L. perenne* which is perennial, and the seeds of the two species are indistinguishable when the lemma of *L. multiflorum* has been de-awned, as often occurs in threshing.

Subsequent investigations have dealt with the genetic behaviour of the factor for fluorescence and its application to seed laboratory practice. Nilsson (327, 328) selfed what was believed to be pure line material of both species of *Lolium* and obtained some fluorescent seedlings from *L. perenne* and some non-fluorescent seedlings from *L. multiflorum.* He considered the character for fluorescence to be recessive and stated that only the Swedish strain, Viktoria, contained non-fluorescent types. As a result, he concluded that the use of ultra-violet light to distinguish between species of *Lolium* was of little practical value. Mercer and Linehan and Linehan and Mercer tested Gentner's method and also investigated the mode of inheritance of the factors responsible for the reaction to ultra-violet light. They found that (*a*) the capacity to fluoresce is heritable, (*b*) *Lolium perenne* seedlings are normally non-fluorescent and *L. multiflorum* seedlings are normally fluorescent, (*c*) fluorescence is dominant in the F_1 generation of the cross between the two species if pure, (*d*) the ratio in the F_2 of fluorescent to non-fluorescent is approximately three to one, (*e*) the ratio of the backcross F_1 by pure non-fluorescent is one to one, and (*f*) there is no genetic linkage between fluorescence and the possession of awns on the lower flowering glumes. Corkill self-pollinated 16 non-fluorescent and 12 fluorescent parent plants. Out of 1,459 seedlings from the former only one was fluorescent. The progeny from nine of the fluorescent parent plants was 100% fluorescent, and from the other three progenies both types occurred which in the F_2 generation gave approximately a three to one ratio of fluorescent to non-fluorescent. The flowering glumes of the non-fluorescent plants were awnless and the leaves conduplicate in the bud, while the flowering glumes of the plants 100% fluorescent were awned and the leaves convolute in the bud. Woodforde's conclusions were the same as those of Linehan and Mercer and of Corkill. He considered that fluorescence is a simple Mendelian dominant and is inherited on a single factor basis. Trumble and Phipps concluded from their investigations that Wimmera ryegrass in southern Australia probably originated as a segregating hybrid from a cross between *L. multiflorum* and *L. rigidum.* The latter is an annual and is non-fluorescent, and the appearance of Wimmera ryegrass was thought to decrease the value of the ultraviolet light test for species of *Lolium* produced in southern Australia. Dorph-Petersen reported that occasional fluorescent seedlings of *L. perenne* were found which were less permanent than the non-fluorescent plants, yet he considered such plants as genuine. The opposite condition was found in strains of *L. multiflorum,* but since such occurrences were rarely noted he concluded that the fluorescence test was a practical method of closely determining the presence of each species in a given lot of seed. Munn (293) studied several species and strains of *Lolium* and concluded that the fluorescense test is entirely useful and extremely practical in evaluating such seed lots. Rampton studied the morphological characters of perennial ryegrass and of strains of domestic ryegrass. The average percentage fluorescence of all strains of domestic ryegrass was 94.2 which was taken to indicate 5% perennial ryegrass in the several populations as a whole. A few lots fluoresced 98% and three lots gave 100%. Nursery readings showed that the domestic strains contained less than 1% of stock resembling perennial ryegrass.

It appears from these several investigations that the fluorescence test cannot be relied upon entirely to determine the exact percentage of true *Lolium multiflorum* and *L. perenne* seeds in all samples at the present time, but its use is of considerable value in that (*a*) pure strains of the perennial type can be detected, (*b*) the minimum percentage of the true perennial type in a lot can be detected, and (*c*) mixed strains labeled as true perennial can usually be detected within 5% or 10% of accuracy. Justice (214) proposed the formula $\frac{\text{“(\% fluorescence–5)} \times \text{\% pure seed}}{\text{\% germination}} = \text{\% Italian ryegrass}$ and hybrids" as a practical procedure in seed laboratories.

An additional use of ultra-violet light in seed technology is employed in some European laboratories to differentiate between strains of yellow and white seeded oats, and Chelmar was able to separate white clover and hybrid clover by the use of the fluorescence test. Gentner tested the reaction of seeds and seedlings of many other species of grasses and other plant families to filtered ultra-violet rays, but no results of practical value were obtained, although a great variation in response was noted.

Chemical Methods. Among the chemical methods employed in seed identification, the picric acid test for cultivated and wild strains of *Trifolium repens* L. deserves special mention. Doak found that the seedlings of wild white clover may be differentiated reasonably well from those of the common white Dutch clover by treating the seedlings with picric acid. The seedlings of the wild form contain a glucoside that hydrolyses at 30° C. and liberates hydrocyanic acid gas which will react with filter paper previously treated with picric acid, thereby producing a brown color. Seedlings of the white Dutch form produce no such reaction.

Another method that has been recently developed is the use of lactic acid as a means of differentiating certain species of *Brassica.* McGugan has found that there is a close correlation between the depths of yellow pigment in the roots and blossoms of adult plants and of cotyledons of certain species of *Brassica.* For example, the seeds of swede turnip (*Brassica napus* L. var. *napobrassica* L. Reichb.) and dwarf essex rape *B. napus* L. var. *biennis* (Schubl et. Mart.) Reichb. are so similar that differentiation by physical features is impossible. The method developed by McGugan is to germinate a mixture of the seeds in the dark for seven days, clip the cotyledons quickly from the hypocotyls and transfer the cotyledons to a filter paper moistened with an excess of a 75% solution of lactic acid. After 12 to 24 hours in the dark the cotyledons of the swede turnip become chrome yellow and those of the rape lemon yellow.

A further adaptation of chemical methods to seed identification is that of the phenol reaction with proteins in wheat. Fraser and Gfeller (143, 144) used a 1% solution of phenol in which mature seeds of wheat varieties were soaked. Among 11 varieties tested, six gave a light brown color reaction and the other five gave a dark brown reaction. By this method they were able, for example, to separate Marquis wheat from a mixture of Garnet and Reward. They also found that the color reactions of spring wheat varieties became intensified as the kernels increased in maturity and in dry matter content. These workers suggested that the method which they developed (*a*) can aid the plant breeder in establishing the genetic composition of hybrids, the identification of pure lines and classification, and (*b*) may be used in commerce for the verification of seed samples, the elimination of synonyms among commercial varieties and the grading of export samples. Waal used the phenol test on Dutch wheat varieties and reported a variation in reaction from colorless to coffee-brown.

Petrova was able to determine mixtures of seeds of Russian and common dandelion by soaking seeds in a 5% solution of either KOH or NaOH and then germinating them. Seedlings of the Russian dandelion were normal, while those of common species were abnormal.

Morphological Methods. Seed analysts have found it necessary to look for morphological differences in seeds of different varieties, species and genera from the earliest beginnings in seed testing because identification was necessary and similarities were common. Such characters of the grasses as length, shape and pubescence of rachilla, presence or absence of awns, point of awn attachment, smoothness or roughness of the lemma, length of the palea in comparison with the lemma, and presence or absence of teeth on the margins of the palea, all were recorded in minute detail and used as aids in seed identification. In other families shape, size and color of seeds, position of the hilum and micropyle and character of the seed coat, whether smooth, pitted, reticulated, grooved or ridged, were observed and noted. Some of the most painstaking and critical work in this field has been done by Hillman with species and varieties of *Agrostis* which are difficult to distinguish even by analysts with many years of experience. Special training is a necessary requirement for accuracy in the identification of *Agrostis* seeds. Hillman's work is a worthy contribution to seed technology. Hillman and Henry prepared detailed drawings of the seeds of over 400 species and varieties of plants which have been of great value in seed testing laboratories. Henry (181) described distinguishing features of certain species of *Agropyron,* and Musil (300, 302–307, 309, 310) has greatly aided seed analysts by her preparation of detailed and enlarged views of seeds of species, long considered difficult to identify, as well as of species of weed and crop seeds not hitherto available to seed technologists. Wright (75) has prepared well illustrated papers for the "Handbook of Seed Testing", covering distinguishing features of seeds of *Setaria, Panicum, Echinochloa, Pennisetum, Poa, Lolium* and *Festuca.* Pepin and Wright found that the seeds of false wild oat have a prominent scutellum above the embryo proper, whereas in the true wild oat this scutellar region is not externally discernible. This means of identification is of value because the true wild oat (*Avena fatua* L.) is classed as a weed. False wild oats (fatuoids) are classed as mutations in cultivated varieties. One basis for this classification is that fatuoids (72, 74) are dormant or non-dormant, according to the variety from which they originate. Murley has recently made a significant contribution by providing seed keys, with clear illustrations, for species and genera of the families Geraniaceae, Euphorbiaceae and Umbelliferae, and Martin (268) and Isely (201) have each provided excellent illustrations of internal seed morphology in many plant families which are of value not only to the seed taxonomist but to the seed analyst as well. Isely (202, 203) has also provided useful seed keys for certain species of *Medicago* and of several genera of the Compositae commonly classed as thistles. Among crop plants several workers (2, 70, 133, 142, 262, 330, 336, 415, 437, 473, 477, 500) have contributed greatly to the identification of varieties by the preparation of well-illustrated bulletins with keys for beans, barley, grape, peas, sorghum, soybean and wheat.

No uniform and constant morphological differences in the external features of seeds within certain species and genera have been discovered, but the seedlings have exhibited distinguishing differences. Recent work in this field has been with botanical varieties and species of *Brassica,* strains of *Trifolium pratense* L., agronomic varieties of *Glycine max* Merr. and horticultural varieties of *Citrullus vulgaris* Schrad.

Seeds of varieties and species of *Brassica* are typically spherical or oval, the cotyledons are folded over on the radicle in a conduplicate arrangement, the size is none too variable, there is some variation in color, and the surface of the seed coat varies from almost indistinguishable to prominent pitting. On the other hand, there are so many botanical varieties within some species, all of which are of considerable economic importance, that differentiation before planting is highly desirable.

The first important approach to the problem of identification of *Brassica* seeds was made by Gorman and Lafferty in 1931. They grew seedlings of swede turnip and rape and discovered distinct differences in the length of the first internode and in the shape of the first leaf. This marked the beginning in a study of seedlings as a means of varietal identification. Hellbo studied the young plants of a number of cultivated species and subspecies of *Brassica* and found distinguishing characteristics. He was not so successful with varieties and strains of the same species. Roberts and Thomas published the results of their studies with varieties and species of *Brassica* and prepared an elaborate key for the identification of seeds and seedlings. The use of their key for seedling identification would require experience and familiarity with the many varieties and species. Robson investigated the seedling characters of some cultivated forms of *Brassica.* He found no constant differences between the seedlings of rutabaga and winter rape, nor between the seedlings of the bulb-forming turnips and their corresponding leafy turnip races. He concluded that when seedlings of winter rape and rutabaga are viewed in mass a fairly reliable guess can be made concerning them, but identification of individual seedlings is not possible. Musil (299) stated that "with practice, it is usually possible to place a seed in one of three major divisions: (*a*) the so-called "swede" group which includes *B. napus* L., winter rape and *B. campestris* var. *napobrassica,* rutabaga (closer to *B. napus* than to *B. campestris*) ; (*b*) the cabbage group, *B. oleracea* L., and (*c*) the turnip, bird rape group (*B. rapa* L., and *B. campestris*), also certain species of the oriental vegetables represented by *B. pekinensis* Skeels, petsai or chinese cabbage. . .". She concluded that the determination of species and botanical varieties within the three divisions is more difficult, and that a growing test to observe the seedlings and older plants is desirable for more positive identification. She gave descriptions and illustrations of the seedlings and leaves from older plants of the following: winter rape, rutabaga, petsai, bird rape, turnip rape, turnip, turnip (strap leaved), spinach mustard, cabbage, cauliflower, broccoli, brussels sprouts, kohlrabi, collards, kale, kale (thousand headed) and kale (dwarf Siberian). The chief points of differentiation, as reported by Musil, for seedlings of the same age are shape, size, color and texture of the cotyledonary leaves, and the angle formed with the stem by the leaves. Similar characters of the first foliage leaves were observed together with differences in the margins of the leaves. The relative length of the stems was also noted as of some value in the identification of plants that were the same age.

The origin of strains of *Trifolium pratense* has become of considerable importance in the United States because the European strains have been shown to be unequal in winter hardiness to the American strains. Inasmuch as the seeds of strains of red clover are indistinguishable, although weed seed content may aid in the determination of origin, it is important to have other means of differentiation. Hollowell found that the seedlings of the European and American strains can be differentiated in that the stems of all European strains have appressed pubescence, whereas the pubescence of the American strains is prominent or rough. This knowledge makes it possible to determine whether lots of unstained red clover seed offered for sale in the United States are native, European or mixed, and is of practical value to American agriculture.

The increased use of soybean in the United States has made it necessary to identify agronomic varieties before planting time. Among the black and yellow-seeded sorts, seeds of several varieties within each color group are so similar that no distinguishing marks exist. Davidson has recently shown that by growing the black seeded varieties Wilson, Otootan, Ebony, Laredo and Peking to the seedling stage it is possible to distinguish one variety from the other. The characters which make this possible are the angle which the cotyledons make with the stem, the curvature, thickness and venation of the cotyledons and the length and color of the hypocotyl. It is not always possible by observation of seedlings to make a positive varietal identification, but it is usually possible to determine whether a lot is not of the variety claimed. This negative evidence is of practical value. Dorchester studied 28 varieties of soybean and found that seedling characters, particularly mesocotyl color, shape of unifoliate leaves and shape of trifoliate leaflets, are a necessary aid to identification.

Recent development of wilt-resistant varieties of watermelon has made it desirable to know some of the characters of seedlings that will aid in varietal identification. Weetman has made a contribution to our knowledge by showing that in one variety the shape of the cotyledons is correlated with melon shape. Seedlings with short or nearly round cotyledons produced round melons, and those with oval cotyledons produced oval or long fruits. These characters may possibly exist in other varieties.

Pathological Methods. The development of disease-resistant varieties of crop plants has resulted in frequent requests by seed laboratory patrons for information as to the identity of a variety with reference to disease resistance. Such requests have come frequently to the Iowa State College Seed Laboratory, particularly concerning varieties of watermelon and oats. The extensive breeding program at the Iowa Agricultural Experiment Station with watermelons for the development of varieties resistant to *Fusarium niveum* E. F. Smith has resulted in the production of seed of new varieties. Occasionally a purchaser wanted to be assured that he had obtained seed of a variety resistant to the wilt organism. Similarly the development by the Iowa Station in cooperation with the United States Department of Agriculture of numerous varieties of oats, resistant to physiologic races of *Puccinia graminis* Pers. and *Puccinia coronata* Corda. and to the species of *Ustilago* that attack oats, resulted in problems of field and laboratory identification because the seeds of certain varieties and strains are almost indistinguishable from susceptible varieties.

The method used by Dr. C. S. Reddy[1] for differentiating varieties of flax resistant and susceptible to *Fusarium lini* Bolley was used with watermelons. The method consists of planting seeds of known susceptible and resistant varieties of watermelon together with seed of the variety to be tested in soil infested with *Fusarium niveum.* It is advisable to treat the seeds of all varieties with a seed protectant before planting because damping-off fungi are usually present in soil, and premature death of seedlings may occur. In addition, seedlings of resistant varieties are sometimes more susceptible to wilt organ-

[1] Outlined to the writer

isms than older plants. The percentage of wilted seedlings in the unknown variety is compared with the percentages in the susceptible and resistant varieties, and thus the unknown is indexed as to its relative resistance or susceptibility. Wilson (490) found that watermelon seedlings from varieties highly resistant to the wilt fungus in the field were often susceptible in the greenhouse if planted in wilt-infested soil. He showed that it was necessary to use soil artificially infested with the fungus so that the concentration of the inoculum could be controlled and standardized. This method of testing varieties of watermelons does not enable one always to determine a variety by name, but it is often possible to determine whether it is resistant or susceptible to *Fusarium* wilt. The method has possibilities in connection with the classification of cabbage varieties for resistance to *Fusarium conglutinans* Woll. and with tomato varieties for resistance to *Fusarium lycopersici* Sacc.

The differentiation of oat varieties resistant or susceptible to the rust fungi can be accomplished by taking the seed of an unknown variety and growing 200 or more seedlings in separate flats. At the same time, seedlings of the known variety, which the unknown is claimed to be, can be grown in flats. Cultures of particular physiologic races of the rust fungi required for differentiation can be propagated on susceptible varieties and transferred to the seedlings of the unknown and the known varieties. After incubation in a moist chamber it is possible to examine the types of reaction and classify the unknown variety as to its degree of resistance and as to the percentage of mixture, if any. The writer, in cooperation with Dr. H. C. Murphy at the Iowa Station, applied this method to several lots of oats with success. To properly use the method in a seed laboratory it is necessary to have access to pure cultures of physiologic races of rust fungi and to have pure seed of known resistant and susceptible varieties of oats. This method could be applied to tests with varieties of wheat and flax when it is desirable to know whether a variety labeled and sold as rust resistant is as claimed. Similarly, the Shamrock cucumber, developed by the Iowa Agricultural Experiment Station from hybrids, made by Porter (348), and combining the resistance of Chinese Long to mosaic virus with the slicing quality of the variety White Spine, can be differentiated from common mosaic susceptible varieties by inoculating the seedlings with the virus of "white pickle mosaic". This method of varietal identification is applicable to many other plant varieties that are resistant to viruses. There is no doubt that the use of pathological methods as an aid in varietal identification can be greatly extended, and thus the tools by which seed analysts more effectively serve those who produce, market and plant seeds will be further increased.

UNIFORM METHOD FOR DETERMINING PURITY OF SMALL-SEEDED GRASSES

The determination of seed purity has long been one of the major objectives in seed testing, and both the Association of Offical Seed Analysts and the International Seed Testing Association have considered the need for uniformity of methods. To that end, referee testing was early adopted as a means of promoting uniformity. Samples carefully drawn from the same lot were submitted to different laboratories with the request that an analysis be made. Comparison of results (1, 54, 358, 397) has shown extreme variation, especially with seeds of *Agrostis, Poa, Dactylis,* and *Bromus,* all of which are classed as chaffy grasses. Until recently no information has been available as to how much variation is to be expected, even in uniform subsamples, and no persistent attempt had been made to determine the causes of extreme differences except to assume that inadequate training of analysts was the major cause.

Extensive investigations (54, 356, 358) have given some information as to how much variation should be expected in well mixed lots of seed of *Poa pratensis* L., *Dactylis glomerata* L., *Agrostis alba* L. and *Phalaris arundinacea* L. when uniform subsamples are drawn and analyzed by a uniform method. The results have shown that differences as reported by official laboratories have been greater than are to be expected when homogeneous samples are tested and, further, that the primary cause for divergent results is inherent in the character of the material involved, in that uniform mixing and bulking is difficult and the similarity and large number of pure and impure particles involved in a working sample provide a great opportunity for variations in personal judgment to operate. For example, one gram of pure seed *P. pratensis* contains from 4,800 to 5,000 seeds, and the physical limitations involved in checking such a number of particles which, on the basis of external appearance, are similar to the pure seed particles, are of some significance. The lemma and palea of each floret prevent a critical examination of the structure enclosed by them, yet an examination is necessary if one is to determine absolute purity. Even if one could examine, under magnification, the contents enclosed by each lemma and palea, uniformity of classification would be impossible by different observers because of gradations in development of the florets as well as differences in personal evaluation.

Brown and Porter (54) in 1935 were the first to publish data which indicated that uniform separation of bluegrass seed is possible by the use of controlled air pressure. They employed a constant speed motor and fan to deliver air through a valve at any given measured opening. Later the junior author (358) showed that the maximum differences in percentage of heavy seed among two sets of 70 subsamples of bluegrass seed when blown by means of an improvised unit and further analyzed in a uniform manner were within a range of two times the standard deviation plus or minus the mean. The mean percentage for one set was 80.5, and the difference in extremes was 7.3. For the second set the mean was 96.6, and the difference in extremes was 2.8. Analysis of the same data by means of the Chi-Square test gave probabilities of .0002 and .0031, respectively, which indicated that either the separation by the air blast was not uniform or the subsamples were not uniform. A third possibility was that the differences in bluegrass subsamples are naturally greater than the Chi-Square test indicated they should be. A subsequent investigation (356) employed a new, single unit, constant speed motor and fan; and with a new type of seed separator, after 64 subsamples of bluegrass seed were analyzed, it was found that the Chi-Square value, as determined from the purity percentages, gave a standard normal deviate of 1.75 which indicated no significant differences. The mean of the 64 percentages was 88.64, and the difference in extremes was 2.37. Uniformity of the light weight material was determined by observing the number of normal sprouts obtained from the separate inert fractions from 32 subsamples. In several trials (356) it was demonstrated by germination tests that pure seed fractions from many subsamples of bluegrass, red top, orchard grass and reed canary grass seed obtained by a uniform method of analysis were remarkably uniform.

Published data obtained from purity analyses of the seed of *Dactylis glomerata* (359, 411) have indicated that a quicker and more uniform method of analysis than the official method is possible. The method is basically the same as that used in the analysis of bluegrass seed, namely, dependance on uniform air pressure for the separation of fertile and empty florets. A modification from the bluegrass method was necessary because the seed of *D. glomerata* contains many multiple florets and the upper ones are frequently empty. It was found that if the heavy fraction was divided into single and multiple florets there was a fairly constant ratio of empty to fertile florets in the multiple group, namely, one to four. In other words, 20% by weight of the multiple floret group in the heavy fraction could be considered as inert, and 80% as pure seed. Analysis of many subsamples from different lots by this method gave uniform results, as indicated by no significant differences in either the percentages of purity or the percentages of germination of 400 seeds from each of the pure seed fractions.

In referee tests Porter (379 [1939: 43–45]) has demonstrated that when a set of subsamples of bluegrass, orchard grass and reed canary grass seed was analyzed by the uniform method of procedure, the results were uniform, but that if the fractions of each subsample were recombined and each one submitted to a separate laboratory the differences were (*a*) not significant if the uniform method of analysis was used and (*b*) significant if the official method was used. Legatt (368) has obtained similar results. In 1937 he (249) used a manometer as a control device to measure pressure of air when samples of bluegrass seed were blown. Leggatt (250) and Porter (356) both designed air blast separators equipped with two devices for measuring air pressure, namely, (*a*) a manometer and (*b*) a dial or gate opening which regulated the flow of air. The latter (356) showed that, with his machine, pressure as measured by the manometer was a safe criterion only when samples of similar weight were used. If samples of unequal weight were used the dial reading was the most reliable criterion, although pressure within the separator tube appeared to be a dependable guide. Aberg *et al.* (1) found that either dial opening or pressure in the blowing tube was a reliable measure of air pressure and that highly uniform separations of heavy and light weight particles of bluegrass and red top seed could be obtained by repeated blowings of the same samples. Porter and Legatt (368) presented convincing evidence of the uniformity of separation by controlled air pressure and demonstrated the practicability of applying the principle to seed testing. More recently Erickson designed a seed blower with the same motor and fan as used by Porter but equipped to handle larger samples.

The logical conclusion from the investigations of methods for determining purity of small-seeded grasses is that previously accepted methods have not given the desired uniformity because of variation in the human factor. Controlled air pressure appears to offer the most hopeful method for a uniform procedure. The use of a uniform method enables analysts to determine whether representative samples have been submitted and whether lots are well mixed and bulked. Finally such an advance in seed technology should eliminate many of the difficulties of seed merchandizing.

Closely related to the problem of uniform procedures in purity analyses is the question of size of sample. Data (379 [1938: 21–24]) obtained from referee tests conducted by the Research Committee of the Association of Official Seed Analysts indicated that one-half gram samples of red top (about 5500 seeds) may be relied upon to give reasonably uniform results, and additional referee tests (381 [1942: 32–33]) with species of *Agrostis* corroborated the earlier data. Aberg *et al.* (1) showed that the use of subsamples of not more than one-half gram in weight gives a reliable measure of the quality of red top seed and further that the variation among one-quarter gram subsamples is normal. A sub-committee of the Association of Official Seed Analysts obtained data (382) which indicated that five-gram subsamples of ***Bromus inermis*** Leyss. give a reasonably accurate measure of seed purity. The use of smaller samples not only reduces the time required but lessens the nervous strain on the part of the analyst.

A PRACTICAL CONCEPT OF PURE SEED

The determination of seed purity with respect to variety or species involves certain problems of classification, especially with broken, injured, shriveled and immature seeds and empty seed cases. The European method provides for the removal from the pure seed fraction of all seeds of the kind under consideration, that are regarded by an analyst as non-viable . Obviously such a method allows for much variation in personal judgment and could hardly be expected to result in a high degree of uniformity. The method that has long been approved by the Association of Official Seed Analysts is that of considering, as pure seed, all crop seeds over half in size, regardless of their condition, whether shriveled, injured or immature. Until recently this rule did not apply to insect-injured seeds, but the Association of Official Seed Analysts in 1940 decided to consider insect-injured seeds as inert when not more than half the embryo is present. This basic method of classification has some merit and has proven of some value for most crop seeds with the exception of those kinds with many broken, insect-injured or diseased seeds or with small-seeded, chaffy grasses, and tobacco seed. Flax, rye, sorghum, soybean and sweet clover seed often contain a considerable portion of broken seeds which are difficult to classify. Seed of Kentucky bluegrass, orchard grass and other chaffy grasses usually contain empty florets which resemble those that contain seeds. Alfalfa, red clover, vetch and numerous other crop seeds are often insect-eaten to such an extent that dissection is often necessary to determine what percentage of the embryo is present. Seed lots of sunflower, cucumber, chicory, endive and tobacco often contain only the hulls or cases of the seeds, yet in external apearance they appear to be true seeds or fruits. All of these partially or completely empty structures from plants are difficult to classify, yet, according to present rules of the Association of Official Seed Analysts, most of them are considered pure seed.

Botanically a seed is a ripened ovule, which implies a completely developed structure capable of reproducing its kind. With the exceptions referred to in the preceding paragraph the method of determining pure seed, as employed by the Association of Official Seed Analysts, has been based partially on the botanical definition of a seed or on absolute purity. Porter (358) showed that determination of absolute purity in bluegrass is a practical impossibility and that when attempted by different seed laboratories results in differences much greater than exist in homogeneous subsamples of seed.

Commercial grass seeds contain heavy, mature seeds, empty florets and various intermediate types, such as badly shriveled or immature caryopses and undeveloped ovaries. The large number of seeds in one gram of pure red top (11,000) and bluegrass (4,800) makes it a physical impossibility to classify all the seeds in such a unit of weight on the basis of absolute purity. An arbitrary, empirical and uniform basis of classification is essential. As was shown in the preceding section, uniform air pressure appears to be the most practical method of separating the empty, immature and shriveled florets, most of which are non-viable, from the heavy, pure seed. The retention in the pure seed fraction of a small percentage of those light weight and mostly non-viable florets causes a decrease in germination that is disproportionate to the increase in purity when such particles are included as pure seed. This statement will be further understood if we consider that tests should provide a basis for the evaluation of seed lots for planting. The most practical evaluation is one which gives either the number of pounds of viable seed per hundred or the maximum number of plants which might be expected under optimum conditions from each pound of seed. Inasmuch as farmers plant largely in terms of pounds per acre and all State and Federal laws adhere to the use of purity and germination in labeling, it would seem more practical at present to determine a figure that will most closely represent the number of pounds of viable seed per hundred, even though variation in the size and weight of seeds of a given kind exists in separate lots.

Porter (360) first called attention to this need for a new concept of pure seed by stating "Laboratory tests of all seeds will more nearly represent the value of seed lots if the unit weight of the living and non-viable seeds in the pure seed fraction is approximately the same. Percentages of purity are based on weight, whereas percentages of germination are based on number. The most practical way in which purity and germination data can be used together is that of determining the number of pounds per hundred of pure, live seed. This may be accomplished by dividing the product of the purity and germination percentages by 100. The retention of light weight, immature and badly shriveled seeds with the heavy seeds increases the difficulty of determining the number of pounds per hundred of pure live seed". The uniform method of determining purity of small-seeded grasses, as shown by Aberg *et al.* (1), commonly gives a purity lower than may be obtained by the official method, but the germination is higher and the product of the two values is greater.

Legatt (253) reached practically the same conclusion. He repeatedly increased the pressure with several different lots of bluegrass seed and made germination tests from the heavy fraction following each blowing. He showed that up to a certain point the increase in germination as it affected the product of the percentages of purity and germination was proportionately greater than the decrease in purity. He designated this point as the "climax point" which is undoubtedly the point at which the unit weights of the living and non-viable seeds in the heavy fraction are practically the same.

Robbins (389) worked with clover and alfalfa seed infested with chalcis fly. He found that a given air pressure removed practically all of the insect-injured seed with the result that the percentage of germination was increased more proportionately than the purity was decreased, and a higher index value resulted.

Present rules for the testing of tobacco seed (430) give an erroneous measure of the quality unless the seeds are cleaned prior to sale or test. Seeds of tobacco are exceptionally small, and many empty or poorly developed seeds are not visibly different from true seeds. Constant air pressure effects a uniform separation of empty and partially developed seeds from true seeds. The line of separation can be adjusted by regulating the air pressure. Empty seeds in samples of grape seeds were removed by flotations in water (335) which no doubt could be done equally well by air pressure. Such empty seeds are not true seeds. Those who have tested seeds of trees refer to empty seeds as a problem in species that normally are dormant. The separation by air pressure should be possible and practicable. Henning[2] has obtained unpublished data with endive and chicory seed which show that the retention of empty seeds in the pure seed fraction of medium to low quality samples gives an index value considerably below the actual value. The latter can be determined by the use of uniform air pressure to remove empty seeds before a germination test is made.

One other aspect of the problem of determining pure seed in seed-testing practice is that of the disposition of diseased seeds. Red-top seed often contains many structures that are partially or completely ergotized. Uniform classification of such particles is impossible by visual methods because of the gradation in infection and the variation in personal judgment exhibited by different analysts. Seeds of rye grass (*Lolium* spp.) are often infected with the blind-seed fungus *Phialea temulenta* Prill. Delcrr. (320). In some cases the caryopses are reduced to very thin light weight structures with only the semblance of an endosperm or embryo. In others the degree of infection is so variable that the line of demarcation between infected and non-infected or viable and non-viable cannot be accurately determined by visual examination. Obviously the only practical method of determining pure seed in lots of diseased seeds is to make a mechanical separation based on specific gravity, and this can be done by the use of uniform air pressure. Separation, by air pressure, of samples of wheat (366) with many shriveled and light weight kernels infected with *Gibberella saubinetii* (Mont.) Sacc. was made into two classes, namely, (*a*) those with a high percentage of infection and a low percentage of viability, and (*b*) those with high vitality and low infection. Flotation of cotton seed in water separated diseased and healthy seed to a practical degree (65). Similarly a practical separation can be made of partially ergotized florets of *Agrostis alba* from healthy seed by air pressure.[3] This method avoids the detailed attempt to classify infected florets by visual examination. Rather, it provides a pure seed fraction in which the unit weight of viable and non-viable particles is the same. Experiments with samples of *Lolium perenne* infected with the blind-seed fungus have indicated that the method of procedure applied to the seed of *A. alba* is equally adapted to seed of *Lolium* species.[3]

---

[2] Unpublished data obtained by Margaret Henning in the Iowa State College Seed Laboratory.

In the Umbelliferae embryoless seeds (fruits) are of common occurrence (141), but the endosperm in such seed is normal. This condition is known to occur also in other plant families and should not be confused with situations where the embryo is removed by mechanical means or by insects and rodents. Any effort to detect embryoless seeds resulting from abnormal development of the ovule is impractical in seed-testing practice and should not be attempted in purity analyses. It is probable that the specific gravity of such seeds is similar to that of normal ones, hence they would be classed as pure seed on the basis of the new concept.

---

[3] Unpublished data obtained by the writer in the Iowa State College Seed Laboratory, Ames, Iowa.

The apparent impossibility of developing uniformity in purity analyses with chaffy grasses, insect-injured seeds, tobacco seed, some vegetable seeds and samples with light weight diseased seeds as well as the incorrect evaluation of seed lots by present official methods and the remarkable uniformity that has been demonstrated when pure seed is largely determined by specific gravity of a particular kind of seed, suggests that the rules for determining pure seed as heretofore employed need revision. The new concept of pure seed as herein described is supported by experimental data which point to the possible adoption of this concept in the practice of seed technology. Its mechanical aspect is the major cause of its high degree of uniformity.

EVALUATION OF WEED SEEDS IN CROP SEED

One of the early objectives in seed testing was to determine the presence of weed seeds. If seeds of objectionable weeds are present in a given seed lot the value of that lot is lowered. Whenever a seed law was enacted. it contained a section which listed the weed seeds that were considered noxious. Sale of crop seed containing seeds of those noxious weeds considered most objectionable was usually prohibited, and in the case of the less objectionable kinds, their presence in excess of a given amount made the sale unlawful. Some seed laws of the present date require that the rate of occurence of secondary noxious weed seeds be shown on the label, and the amount is limited only by the total weed seed allowance stipulated in the law.

In the early development of seed testing no effort was made to evaluate the condition of weed seeds or fruits. Regardless of their stage of development, they were classed as weed seeds and so listed on reports of tests. For example, fruit structures of *Rumex crispus* L. and *R. acetosella* L. with only a pericarp and seed coats, but with no embryo or endosperm, were classed as weed seeds. Empty lemmas of *Agropyron repens* Beauv. and other weedy grasses, and fruit structures of *Cirsium arvense* Scop. with no embryo, caused either by failure to develop or by insect injury, were also classed as weed seeds. Similarly a fruit of a weedy grass from which the embryo had been removed by an insect or by mechanical injury was considered a weed seed.

As the menace of weeds increased in agricultural regions and improvements in seed cleaning machinery were affected, the requirements in seed laws relative to the presence of noxious weed seeds became more stringent. At the same time the difficulties of removing certain weed seed structures of a given size or weight without a severe reduction in the quantity of salable seed, led to a more scientific evaluation of the significance of weed seeds in crop seed.

One of the early papers which dealt with the viability of weed seed in crop seed was by Stahler in 1935, who reported that hulled seed of *Agropyron repens* germinated from zero to 25%. He made no classification as to presence or absence of the embryo. The following year Porter and Koos published data on the relative germinability of normal, injured and hulled seeds of *Rumex crispus, Polygonum Convolvulus* L., *Chaetochloa lutescens* Weigel (Stuntz), *C. viridis* L. (Scribn.), *Ambrosia artemisiifolia* L. and species of *Cuscuta.* They reported that in all cases the injured or hulled seeds germinated much less than normal seeds or fruits. Pale colored dodder seeds, irregular in shape or enlarged abnormally, were non-viable. Wright (497) reported on tests of hulled seeds of *Ambrosia artemisiifolia* and *Agropyron repens* that were conducted in several Canadian Seed Laboratories. He considered that 10 hulled seeds of *A. artemisiifolia* had a value equal to one normal fruit in viability and that injured embryos of *A. repens* possessed very low vitality. Later, in 1938 (498), he made a report to the International Seed Testing Association in which he proposed that weed seeds be classed as inert matter when they are completely crushed or when the embryo is visibly missing.

In 1937 the chairman of the research committee of the Association of Official Seed Analysts at the Annual Meeting of the Association in Washington, D. C., proposed that seed or fruit structures of weeds known to be incapable of germination, such as embryoless fruits of weedy grasses, sterile dodder seeds and empty glumes of weedy grasses, be classed as inert matter. After much discussion the Rules Committee prepared a paragraph for inclusion in the revised rules which stated that weed seeds, which by visual examination are considered incapable of germination, should be classed as inert material. In that same year a sub-committee of the research committee was appointed to study specifically the viability of weed seeds in crop seed. This sub-committee aroused much interest among the members of the Association, and a number of previous contributions were reviewed and additional ones made. The contributions which followed were published in the Proceedings of the Association of Official Seed Analysts (217, 384) from 1938 to 1944. In 1946 Justice and Whitehead reported that a very low percentage of the fruits of *Cyperus rotundus* contain embryos; hence the majority of what appear to be fruits are inert material. The data that have been obtained deal with various types of injured and undeveloped seeds in comparison with normal seeds or fruits from the following plants:

Agropyron repens
Allium canadense L.
A. vineale L.
Amaranthus spp.
Ambrosia artemisiifolia
Avena fatua
Bromus tectorum L.
Centaurea solstitialis L.
Chaetochloa viridis
Convolvulus arvensis L.
Cuscuta spp.

Cyperus rotundus
Digitaria sanguinalis L. (Scop)
Helianthus annuus L.
Holcus halepensis L.
Plantago lanceolata L.
Plantago spp.
Polygonum aviculare L.
P. convolvulus
P. persicaria L.
P. scandens L.
Rumex crispus
R. obtusifolius L.
R. salicifolius Man.
Salsola kali L.
Thlaspi arvense L.

As a result of this extensive study it was possible for the Special Committee on Standardized Tests to prepare a list (380) on specific weed seed structures that should be classed as inert material rather than as weed seeds. The recommendation made by this committee was approved and incorporated in the rules for seed testing adopted in 1944. Later the United States Department of Agriculture incorporated the recommendation in its rules for seed testing. The specific section dealing with this recommendation lists the structures from weed plants that are to be classed as inert material, namely,

> All badly injured, undeveloped or empty structures which resemble seeds but which by visual examination (including dissection or reflected light) can be easily demonstrated as having no embryo or having only a rudimentary

embryo that has been destroyed by a disease organism. Included in the category of inert matter are; (a) seeds of grasses with over half the embryo removed, (b) seeds of dodder which are usually fragile, ashen gray to brown in color and somewhat enlarged, (c) ragweed seeds with both the involucre and pericarp absent, (d) shriveled, blackened seeds of buckhorn, (e) "empty seeds" or fruits, such as occur in the sedge, buckwheat, morningglory, and sunflower families, (f) empty glumes and sterile florets of grasses, (g) naked seeds (seed coats absent) of the species of *Brassica* usually considered as weeds and of *Leguminosae,* (h) bulblets of wild onion and garlic with the basal or stem end portion removed. (An air blast separator will often be an invaluable aid to the initial separation in classes b, c and f.)

The adoption of this procedure in the classification of weed seeds marks a further step in advance in seed technology and has helped to place another phase in the practice of seed testing on a more scientific basis.

MORE UNIFORM EVALUATION OF SEED GERMINATION TESTS

Seed germination is defined in botanical text books as "sprouting of the seed" or "resumption of growth by a dormant embryo". Neither definition suggests how far the sprouting or growth must proceed to be classed as germination. In early attempts to determine germinability, especially before seed scarification was practiced, a seed which produced a primary root, a primary stem, or both, after a given number of days was considered to have germinated. No special attempt was made to evaluate the young seedlings in terms of their ability to produce plants. Blotters were most commonly used as the substrate for germination tests, and at the end of a given period the number of sprouted seeds between the folds of a moist blotter was determined.

In the European laboratories the removal from the pure seed fraction of all seeds considered incapable of germination greatly reduced the number of abnormal growths and thus simplified the evaluation of the seedlings. In the Amercian laboratories the retention of broken, injured, shriveled and immature seed in the pure seed fraction from which seeds were drawn for the viability test resulted in many broken or otherwise abnormal seedlings whose value in crop production was open to question. The development of methods for the scarification of impermeable seeds also accounted for many broken sprouts among seeds of *Melilotus alba* Desn. and *Melilotus officinalis* Lam. As a result it became necessary to develop criteria for the evaluation of seedlings.

Early efforts to classify sprouted seedlings on the basis of estimated ability to produce plants resulted in extreme variation of opinion among analysts which has been a source of great difficulty in seed merchandizing both within and between countries. For the past 20 years referee tests by both the Association of Official Seed Analysts of North America and the International Seed Testing Association have demonstrated that differences in interpretation are far beyond the realm of normal variation in homogeneous seed lots. Furthermore only partial agreement has existed among analysts as to what constitutes normal and abnormal seedlings. In recent years it has become increasingly evident that two fundamental needs existed, namely, standard media for the germination of different kinds of seed and a standard guide for classifying seedlings as normal or abnormal.

The Association of Official Seed Analysts in its "Rules for Seed Testing", approved in 1928, advocated the use of soil as a medium for seed germination in which to determine normal seedlings. In general, steam autoclaved soil or soil found to contain no pythiacious or other "damping-off" fungi was recommended. Soil was recommended in preference to sand because the former was considered a natural medium for seed germination, although autoclaved soil has lost many of its natural characteristics.

The use of soil as a medium which offered resistance to the emergence of seedlings was discussd by Brown and Toole and Munn (294). This attempt to develop an emergence test as a measure of normal seedlings marks a forward step in the field of seed technology. The wide difference in soils and the possible chemical and biological changes resulting from treatment of soil in an autoclave were naturally not adequately recognized in the early work with substrate for seed tests. Furthermore personal interpretation of normal and abnormal seedlings among those that emerge in soil has been too variable; hence the desired uniformity that was anticipated did not materialize.

Within the past decade or more considerable emphasis has been placed on the use of sand as a seed germination medium. Deming and Robertson in 1933 used sand as a means of determining dormancy in the seeds of wheat, oats and barley. Leggatt (379–1935) in 1934 prepared a special quartz sand of a given particle size, washed it with dilute hydrochloric acid and studied its value in seed germination. He concluded that it is highly satisfactory as a substrate. The Research Committee of the Association of Official Seed Analysts (379–1936) in 1936 initiated referee tests with sand in comparison with blotters and soil, and in 1937 a sub-committee was formed to give special attention to the use of sand as seed germination medium. Since that time many reports of the Research Committee (379–1938: 38. 1939: 46–51, 383) have included tests with quartz sand and different grades of local sand that were available to cooperating laboratories. The net result of these studies was the development of a standard method of procedure for testing seeds of various kinds of plants. Reference to this standard will be made subsequently.

Dunlap used clean sand to which nutrient solutions were added as a medium for seed germination. He considered it superior to soil and found that it controlled damping-off successfully. Dunlap and McDonnel used sand in pots that were set in water to test seed for germination. The sand absorbed enough water to provide moisture for gemination. Erickson and Porter compared the germination of soybean seed in the field with that in sand and soil in the laboratory. They found that in several cases field germination was higher than in laboratory soil but never higher than in laboratory sand. Porter (362) made a subsequent comparsion of field germination with laboratory germination in towels, sand and soil using 100 lots of soybean seed. He found that the coefficient of variability for laboratory sand and field tests was 0.83 for the 1936-crop seed and 0.72 for the 1937-crop seed. The correlation between soil in the laboratory and the field was lower for both classes of seed. In no case was germination in sand lower than in the field, whereas germination in towels and in soil was frequently lower than in the field. These latter results indicated that towels and soil give uncertain results because laboratory conditions should be more favorable than field conditions. Eliason and Heit reported that sand is superior to the Jacobsen germinator in detecting injured seed in lots of red pine seed. Davis (100) considered sand of value for germination of cucurbit seeds. Porter (358) recommended sand in petri dishes as a medium superior to blotters for the germination of seed of Kentucky bluegrass. The same method (362) was tested for seeds of orchard grass and reed canary grass with satisfactory results using "Flint Silica" or "Black Hawk No. 2" quartz sand. As a result of extensive trials in the

Iowa State College Seed Laboratory the use of sand in long benches was adopted as the most practical and satisfactory medium for the testing of seeds of corn, cowpea, soybean, garden bean, pea, pumpkin, squash, watermelon, cantaloupe, cucumber, horse bean, barley, oats, wheat, rye and sorghum. Builder's sand be can used several times in succession if it is heated to 80° C. for 15 to 20 hours. (362).

Materials other than sand for germination of seeds have received some attention. Hope *et al.* recommended living or commercial sphagnum moss as a substrate for many vegetable seeds; they also reported that they obtained good control of damping-off and that transplants were easily made to pots or flats. Stuart tested an expanded vermiculate ore sold under the name of "zonolite" and recommended it highly as a medium for seed germination. He reported that it drains well, holds water and air satisfactorily, can be sterilized for subsequent use, and that nutrient solutions can be added. The practical application of these two materials to seed testing on a large scale is yet to be determined, but they undoubtedly have possibilities. It is possible that as our knowledge of the requirements for seed germination increases we may find it expedient to use a completely inert material to which nutrient solutions will be added according to known requirements.

The second major problem in the standardization of germination testing, namely, development of criteria for evaluating seedlings, has likewise received attention. It is now generally recognized that the primary purpose of a seed germination test is to determine the ability of seeds to grow and produce normal plants. It follows, therefore, that one must know the requirements for the germination of each particular kind of seed and be able to evaluate each seedling in terms of its plant-producing power. Two pertinent questions may well be raised: (*a*) Must the requirements be such as will measure maximum viability? (*b*) What is a fair measure of plant-producing power? Seed technologists must not only answer these questions but also agree on a set of rules which all can follow.

The great variability in the physical and chemical condition of soils in which a given lot of seed may be planted, as well as differences in temperature and moisture content, are so obvious that reference to them seems almost unnecessary. Yet these variations will cause a marked difference in field germination and plant survival of a particular lot of seed. Many investigators have compared the laboratory and field germination of seeds. In numerous tests (132, 351, 366, 433) field germination has equalled that in the laboratory, even when optimum conditions were provided. This has been particularly true with seeds of corn, wheat, oats, barley, peas, beans and soybeans. In other cases (364) field germination has been much lower than that in the laboratory but only when field conditions have been decidedly unfavorable. Small-seeded vegetables and field crops, such as onion, carrot, cabbage, clover, alfalfa, bluegrass and red top, may be expected never to germinate as well in the field as in the laboratory unless exceptional care in seeding and watering is taken with the field planting.

Differences among kinds of seeds in their ability to produce plants in the field under varying conditions are considered by agronomists and horticulturists when they recommend rates of seeding based on a standard percentage of germination. Seed technologists, therefore, have to decide whether their standard for measuring viability is to be optimum conditions for germination or less, and if less, how much. The obvious answer is "optimum" as evidenced by the repeated and continuous efforts of seed investigators to find how to germinate many kinds of seeds. Optimum conditions produce maximum germination which has come to be recognized as the measure of seed viability, even though field germination may rarely equal the laboratory maximum.

The real problem is that of evaluating the seedlings that result from a laboratory germination test. First of all it has been necessary to define germination in terms of seed laboratory practice. The Association of Official Seed Analysts and the United States Department of Agriculture in their "Rules for Seed Testing" have from time to time attempted to formulate a definition. In1928 the Rules stated: "Since we wish to obtain as a result of the germination test, the percentage of seeds capable of developing into normal seedlings, only such seeds should be counted as germinated which may reasonably be expected to continue their development under favorable conditions". In 1941 the writer proposed a definition to the Iowa Legislature which was incorporated in the Rules and Regulations of the Iowa Seed Law (399) as follows: "A seed shall be considered to have germinated when it has developed those structures that are considered essential for a normal seedling. Broken seedlings and weak, malformed and obviously abnormal seedlings shall not be considered to have germinated". In 1944 (380) the Committee on Standardized Tests of the Associaton of Official Seed Analysts adopted the following definition of seed germinaton: "In seed laboratory practice, germination is defined as the emergence and development from the seed embryo of those essential structures which for the kind of seed in question, are indicative of the ability to produce a normal plant under favorable conditions". This definition was approved and adopted by the Association of Official Seed Analysts and by the United States Department of Agriculture.

The preceding definition of seed germinaton grew out of a careful study by several seed technologists and other workers with various types of seedlings produced by different kinds of seeds planted in several types of substrate. Workers in the United States Department of Agriculture made significant contributions by growing seeds in sand, soil, blotters, petri dishes, towels, as well as in greenhouse flats and in the field. They made photographs (13) of normal and abnormal seedlings of a large number of kinds of plants, all of which were studied in detail by the Committee on Standardized Tests and compared with actual seedlings that had been grown by members of the Committee. As a result of this cooperative study is was possible for the Committee to prepare a report (380) which described both the essential structures of normal seedlings in a large list of crop plants and the standard medium in which the seedlings should be grown in order to classify them by the approved standards. Porter (362) outlined criteria for evaluating seedlings, described the media in which they could be grown, and illustrated by drawings the essential structures of normal seedlings as well as characteristics of many types of abnormal seedlings.

One of the most perplexing problems was the evaluation of baldhead seedlings in beans described by Harter and Borthwick. Drake (119) made a laboratory and field study of "baldhead" seedlings and concluded that they should be classed as abnormal seedlings. A more recent report (120) of work with "baldhead", "snakehead" and "half-snakehead" seedlings showed that the yield of such plants is decidedly inferior to that of normal plants. Ingalls, Nutile (333) and Wester studied the yield of normal and "baldhead" plants of beans and were in general agreement with Drake as to their evaluation in field plantings.

The development of these standards in seed germination and in the interpretation of the results marks a great step in advance in the practice of seed technology. Further developments are basically dependent on advances in our knowledge of the optimum requirements for the germination of many kinds of seeds. Species of wild plants are being added frequently to our list of economic plants, and as a result it becomes necessary to study the germinability of their seeds. In addition new information concerning the germinability of species long considered of economic importance has been made, available. Subsequent chapters will deal specifically with dormant and impermeable seeds. In this chapter it is necessary to refer to only a few papers that deal with environmental conditions which facilitate germination.

The effect of soaking seeds prior to germination and of alternate wetting and drying has received some attention. Germination of seeds of *Dactylis glomerata* (68) was considerably accelerated by soaking the seeds in water and drying them at any time prior to placing them under germinative conditions. The reason advanced was that treated seeds absorb water more rapidly than normal seeds, the paleas of which are at first impermeable. In a study of 42 species of range plants from Utah (156) it was found that the behaviour of seeds subjected to rapid and slow drying depended upon the species, the rate of drying and the time when drying occurred. Drying hastened and increased the percentage germination of some species, while in others germination was delayed and decreased. In a few species the effect of drying was negligible. Seeds of *Buchloe dactyloides* (Nutt.) Engelm. (484) soaked 48, 72 and 96 hours in water averaged 59.5% and 80% germination, respectively, on a water and a 0.2 percent KNO_3 substrate, whereas unsoaked seeds gave 8.6% and 33% on the same respective substrates.

Germination requirements for seeds of several species and varieties of *Festuca* were found to be variable (221). Freshly harvested and immature seed required a low temperature, but as the seed aged it germinated over a wider range of temperatures. Seeds of *Festuca rubra* germinated best at 10°C., while other species and varieties germinated at higher and alternating temperatures. The requirements for germination depended on age and storage temperature and humidity. Favorable response to light and treatment with KNO_3 at an optimum temperature was exhibited only by seed of *Festuca capillata.* Tap water sometimes contains chemicals that are injurious to germination. Addition of calcium to distilled water (47) retarded the germination of seed of *Hypericum perforatum* L. to the same extent as did tap water. It was considered that high alkalinity of tap water is not a factor, provided calcium is absent. It was suggested that seed coat impermeability may be altered in the presence of calcium. It is known that chlorine in tap water is injurious to the germination of some seeds; hence distilled water is required.

A study of the effect of temperature on the germination of seed of *Astrebla lappacea* Domin. (314) revealed that a constant high temperature (33°C.) was more favorable than constant low (20° or 16°C.) and equal to alternating temperatures which usually are favorable to seeds of many grasses. No difference in the effect of light or darkness on seed germination was observed. Heit (173, 174) has recently summarized data obtained from the germination of more than 100 flower seed species by different methods.

The accumulation of carbon dioxide in certain types of germinators is known to be injurious to seed germination. A recent study of seed germination in carbon dioxide is of interest (456). At seven temperatures ranging from 5° to 35° C. it was found that from 40% to 80% CO_2 was necessary in the presence of 20% oxygen to inhibit germination of the seeds of cabbage, pepper, pea, radish, sunflower, tomato and wheat. Seed germination of buckwheat, delphinium and onion was inhibited under the same conditions only at 10°C. Seeds of cabbage, pea, sunflower and wheat initiated germination at all concentrations of CO_2 and at all temperatures except 5° and 10°C. where high concentrations inhibited all but cabbage seed. The inhibiting effect was greatest at low and least at intermediate temperatures. The need for oxygen in seed germination is basic with most seeds, however; some seeds germinate in almost complete absence of oxygen. Seeds of *Peltandra virginica* (L.) Kunth. and of rice (126) germinated with little or no oxygen. The coleoptile of *P. virginica* elongated two to three times its original length due to elongation of the cells already formed in the embryo.

ADVANCES IN OUR KNOWLEDGE OF IMPERMEABLE SEEDS

The chief developments in this field have dealt with (*a*) cause of impermeability, (*b*) relation of seed maturity to impermeability, (*c*) effect of storage conditions on impermeability, (*d*) treatments for impermeable seeds, and (*e*) value of impermeable seeds in crop production.

The most significant contribution made within recent years regarding the nature or cause of impermeability is by Hamly. He discovered that uninjured seeds of *Melilotus alba*, known to be permeable, when soaked in a 1% solution of osmic acid develop a blackened area on the side of the hilum opposite the micropyle. This area is known as the "strophiole". When such seeds were sectioned it was found that from the strophiole the darkening extended through a cleft in the columnar cells of the Malpighian layer to the embryo, following a path which would be taken by water upon its entrance into the seed. Impermeable seeds showed no strophiolar discoloration and when sectioned there was no cleft between the Malpighian cells. Hamly found further that suberin, the principal constituent of the caps of the Malpighian cells, causes a sealing of the seed coat in mature impermeable seeds. He also reported that the light line is an optical illusion and not associated with impermeability. These phenomena were first detected in a lot of sweet clover seed that had been passed through the Eddy Huller and Scarifier which is equipped with carborundum discs against which the seed is thrown. Mature seeds that were 99.5% impermeable were then placed in a 500 ml. Florence flask and shaken for 10 minutes at three oscillations per second. The permeability was increased to 91% and the permeable seeds became blackened at the strophiole in osmic acid. Hamly's explanation of his results was that by impaction the seeds receive one or more blows at the strophiolar region sufficient to separate the Malpighian cells through which water could enter. A similar condition was found in seeds of *Trifolium pratense*.

Martin (270) concluded that no true strophiole occurs in sweet clover and alfalfa, the palisade layer is doubled and arched near the micropyle, but no openings, excresences or tubercles were found about the hilum. In a subsequent paper Martin and Watt agreed with Hamly that initial water absorption by sweet clover seeds occurs normally through the strophiole. Hutton and Porter reported that impaction of impermeable seeds of *Amorpha fructicosa L.* and *Lespedeza capitata* Michx. cause fissures to appear at the base of the hilum depression, through which water may enter and bring about germination. Shaking seeds of these two species for 10 minutes in a glass bottle reduced the impermeable seeds to zero. Simpson *et. al.* (424) found that areas in the micropilar and chalazal regions of cotton seed are especially adapted as absorptive tissues, that final entrance of water is primarily through the chalazal opening and the micropyle, and that variations in different seed lots account for differences in the rate of germination. Preston and Scott studied seed development of *Vicia Faba L.* and *Phaseolus multiflorus* Willd. and concluded that the micropyles become blocked during development. Absorption of water through the microplye of permeable seeds was significantly higher than through holes bored in the seed coats.

Little work has been done on the relation of moisture content of developing seeds to their impermeability. Watt found in *Melilotus alba* that the light line appeared within 16 days after pollenization and that the seed coat was mature in 20 days. Helgeson classified immature seeds of *M. alba* into "yellow" and "brown pod" groups and found that the moisture content of both groups was higher than that of ripe seed but that the "yellow pod" group had more than the "brown pod" group. Seeds of the "yellow pod" group germinated 100% in 10 days, while those of the "brown pod" group and ripe seeds contained 43% and 86.5% impermeable seeds, respectively. Raleigh mentioned that impermeability of the seed coat of *Gymnocladus dioica* (L.) Kock. seems to be caused by a general shrinking and condensation of the seed coat in drying. Middleton (283) showed that large and small seeds of Korean lespedeza contained 15.9% and 37.4% impermeable seeds, respectively.

Tests to determine the relation of moisture content to germinability and impermeability of seeds have been made (195), using seeds of *Strophostyles helvola* (L.) Britton. Seeds with 57.87% moisture germinated 64% with no hard seeds, those with 43% moisture germinated 100%, and those with 20.18% moisture germinated 48.5% and contained 51.5% hard seeds Seeds of *Convolvulus arvensis* L. were harvested at different stages of maturity (55). Seeds with 81% moisture were able to germinate at once, and when the moisture content was reduced to 13%, impermeability developed.

The effect of storage conditions on impermeable seeds is very marked in some species, depending on the temperature and humidity of the storage environment as well as on the length of storage. The percentage of impermeable seeds in sweet clover was reduced only slightly after 11 years of dry storage, according to Stevens and Long. Storage of alfalfa seed for one year under laboratory conditions was sufficient to bring about permeability to all impermeable seeds, according to Lute. Jones was able to reduce the amount of impermeable seeds of vetch (209) by storage in a humid atmosphere. Middleton (282), who studied the seeds of *Lespedeza stipulacea* Maxim., discovered that the hard seed content declined from 47% in November to 11% the following March and that the percentage of germination increased from 46.7 to 84.8 in the same period. The seeds were stored in a laboratory room at Raleigh, North Carolina. Helgeson made extensive studies with seeds of *Melilotus alba.* He found that immature seeds were practically all permeable, that this condition could be prolonged for 19 months in a moist cool room and that dry storage induced impermeability. Storage out-of-doors reduced the percentage of impermeability in both mature and immature seed lots. Seeds of *Strophostyles helvola, Lespedeza virginica* (L.) Britton and *L. capitata* were stored for two years in a laboratory room at Ames, Iowa (195). At the end of the period there was no significant change in the percentage of normal seedlings, but there was a marked increase in the percentage of dead seeds and a corresponding reduction in percentage of impermeable seeds except for *L. virginica.* Presumably some of the seeds that were permeable at the beginning of the period died, while some of the impermeable seeds became permeable and retained their vitality, but for both species of *Lespedeza* the percentage of germinable seeds at the beginning and the end of the period was so small that most of the seeds that became permeable during the storage time must have lost their vitality.

Working with seeds of *Melilotus alba* and *Vicia villosa* Roth., Dunn kept samples of each for one to ten months in moist and dry storage at temperatures of 5° C. and -10° C. No low temperature storage treatment was found that would cause samples to give higher germination percentages than seed samples which had been stored dry at room temperature. Moist, dry and alternating treatments caused softening of the hard seeds of *Vicia* but not of *Meli-*

lotus. After six months storage only 20% of the original hard seeds of *Vicia* were still impermeable. Martin (271) reported that storage of seeds of *M. alba* at fluctuating temperatures in the realm of freezing for two or more months or in unheated open buildings during the winter provided the most favorable conditions for softening. No softening occurred in seeds of *Lathyrus maritimus* (L.) Bigel. after 11 months of storage (110).

Seed lots of *Desmodium tortuosum* (S.W.) D. C. (213) containing from 4% to 82% hard seeds lost from 0% to 26% in viability over a period of 18 to 19 months, depending on the quality of the seed. The sample with 82% hard seeds showed no loss, but after treatment with sulfuric acid and storage for six months there was a decline of 11% viability.

Treatments of impermeable seeds within the past 20 years have included high pressure, low temperatures, wet or dry heat, soaking in sulfuric acid, scarification and impaction. Davies (97) increased the germination of hard seeds of *Medicago sativa* L. and *Melilotus alba* 30% by exposing them to pressures ranging from 500 to 2,000 atmospheres for five and ten minute periods. More recently, Rivera *et al.* made an extensive study of the effect of pressures as high as 60,000 pounds per square inch on impermeable seeds of several species of plants. Little difference was noted between treated and untreated seeds of *M. sativa* and *M. alba* with respect to germination, but pressures up to 30,000 pounds brought about germination of impermeable seeds of *Cladrastis lutea* (Michx.f.) Koch. Pressures of 45,000 pounds or more were injurious. The most favorable temperature for treatment was 25° C. Seeds heated to 50° C. or scarified failed to germinate. Seeds of *Gymnocladus dioica* germinated only after exposure to a pressure of 1,000 pounds at 50° C. A pressure of 10,000 pounds at 25° C. caused the highest rate and final percent germination of the seeds of *Ditremexa occidentalis* Britton and Rose. All pressure treatments were effective on seeds of *Roystonea regia* O. F. Cook but ineffective on seeds of *Hamamelis virginiana* L., *Cornus florida* L., *Crataegus mollis* (T. and G.) Scheek and *Rosa* spp. Long exposures to 10,000 pounds per square inch increased the formation of acids in seeds of *C. lutea* and *Ricinus communis* L.

Midgley used alternate freezing and thawing with impermeable alfalfa seeds and found that the first freezing made the seeds permeable, but subsequent freezing was of no avail. Dry and wet freezing in a humid atmosphere was equally effective. Busse froze seeds of sweet clover and alfalfa in liquid air. A temperature of –80° C. made seeds of alfalfa permeable, but –190° C. was required for the same effect on hard sweet clover seeds. Seeds of *Lespedeza capitata, Strophostyles helvola* and *Amorpha fruticosa* (195) were frozen at –6.5° C. for 16 hours, thawed and refrozen again for 8 hours. This treatment had no effect on the seeds of *L. capitata* and *A. fruticosa* but reduced the percentage of impermeable seeds slightly and killed most of the permeable seeds of *S. helvola.* Dry heat was applied to alfalfa seed (264) at temperatures of 60°, 80°, 85° and 90° C. Treatments for over four hours at 85° or 90° C. were injurious, but shorter periods increased the germinability. ✓

Using wet heat by placing seeds in a water bath at temperatures of 65° and 85° C. for one to eight hours, Hutton and Porter immersed seeds of the same species listed in the preceding paragraph. Exposures up to three hours at 85° C. had no effect on permeable or impermeable seeds of *Lespedeza capitata,* but a five-hour exposure reduced the percentage of hard seeds and increased the percentage of dead seeds of *Strophostyles helvola.* One or two hours at 85° C. reduced the percentage of impermeable seeds of *Amorpha fruticosa* and two hours increased the percentage of dead seeds. Dry treatment at 85° C. for one, two or three hours increased the percentage of impermeable seeds of *A. fruticosa.* Harding recommended immersion of seeds of *Acacia pycnantha* Benth. and *A. acuminata* Benth. in boiling water for five seconds as the most satisfactory treatment, and Wilson (491) found that a similar treatment for one minute was effective for seeds of *Robinia pseudo-Acacia* L., *R. hispida* L. and *R. viscosa* Vent.

Sulfuric acid has long been employed as an agent for the treatment of impermeable seeds. Many species of seeds, hitherto not investigated, have been included in recent work, primarily because of the importance of the Leguminosae in soil improvement and conservation, although members of other families have been included. Rogers, working with *Amorpha nana* Nutt., hastened germination by several days and increased total germination by soaking seeds in concentrated H_2SO_4 for five to seven minutes. Seeds of *Gymnocladus dioica* have an exceptionally hard seed coat and are slow to germinate in field soil. Wiesehuegel obtained 86%

germination by soaking the seeds two hours in concentrated H_2SO_4. Meginnis considered that treatment of the seed of *Robinia pseudo-Acacia* in H_2SO_4 was superior to immersion in hot water. He obtained twice as many seedlings in the field from acid-treated seeds as from the controls, although he found it necessary to vary the time of soaking according to the variation among seed lots. A preliminary test of small samples was recommended. Peneff reported that the best treatment for this species was one and a half to two hours which tripled the rate of germination. Treatment of seeds of *Albizzia acle* Merr. (106) with H_2SO_4 for 60 minutes was necessary to bring about germination, and the optimum immersion period for seeds of the Cuban fiber plants, *Triumfetta semitriloba* and *Urena lobata* (189), were 15 and 60 minutes, respectively. Immersion of seed of *Strophostyles helvola* for 30 minutes in concentrated H_2SO_4 was the only satisfactory method of treatment that was attempted (195). A seed lot with 57.6% hard seeds and a germination percentage of 29 gave 79% normal seedlings with 3% hard seeds following the acid treatment. Steinbauer (435) tried 14 treatments on seed of the sweet potato and concluded that concentrated H_2SO_4 for 20 minutes was the most reliable.

Use of concentrated H_2SO_4 on the seeds of *Convolvulus arvensis* L. (55) for 30 minutes was necessary to soften practically all of the impermeable seeds. Treatment of the seeds of *Leucaena glauca* Benth. with H_2SO_4 increased the germination from 15% to 90%, according to Akamine (5), and Musil (308) obtained complete germination in three to six days by treating seeds of *Cyamopsis tetragonolobus* S. Taub. for 15 minutes with 75% H_2SO_4. No injury resulted from a 30 minute treatment. Treatment of seeds of *Lathyrus maritimus* was made by Dinnis and Jordon who recommended immersion for 90 minutes in concentrated H_2SO_4. Lemmon *et al.* worked with *L. maritimus* and *L. littoralis*. They concluded that treatments for 20 to 30 minutes, respectively, of the two species in H_2SO_4 were sufficient and that the savings in seeding cost per acre were 39% for the former and 24% for the latter. Furthermore, treated seed stored for as long as 197 days was still as germinable as immediately after treatment. Justice and Marks reduced the impermeability of seed of *Lathyrus hirsutus* L. from 70% to 0% by immersing the seed in concentrated H_2SO_4 for one hour. The optimum period of immersion varied from 30 to 60 minutes with different samples.

Scarification of seed by the use of abrasives has been used extensively by many workers with varying results, although in general most machines cause considerable injury which results in a breaking of the hypocotyl from the cotyledons. Those unfamiliar with seedling structures have sometimes failed to recognize such injuries in their evaluation of scarification methods. Reduction of impermeability with resultant abnormal or broken sprouts is of little value and may provide a seed lot of less value than before treatment.

Wiesehuegel used a file on seeds of *Gymnocladus dioica* and obtained 80% germination. Hutton and Porter, by rubbing seeds of *Amorpha fruticosa* lightly with sand paper, reduced the percentage of impermeable seeds to zero without any increase in dead seeds or in abnormal sprouts. When seeds of *Robinia pseudo-Acacia* were placed in a revolving drum with glass splinters and rotated for 30 minutes at 50 to 52 r.p.m., Peneff obtained 100% germination. Scarification of seed of *Leucaena glauca* gave 90% germination, according to Akamine (5), and Sell found that scarification of bur clover (*Medicago* sp.) after hulling was the only satisfactory treatment. He obtained 100% germination in two weeks. A seeding rate of 72 pounds per acre of untreated seed produced fewer plants than 1.3 pounds of scarified seed. Martin (269) was able to obtain a much higher percentage of sweet potato seedlings from acid treated and scarified seed than from untreated seed of six varieties representing seed with nine months and four years of age, respectively. For large quantities he recommended soaking 45 to 60 minutes in concentrated sulfuric acid. Germination of *Lupinus* seeds was induced by seed coat removal and of *Baptisia* by scratching the seed coat, according to Burunjik.

Reduction of seed impermeability in seed lots of *Melilotus alba* and *Trifolium pratense* by impaction, as discovered by Hamly to which reference has been made, has received limited attention and trial. Porter (352), three years after the publication of Hamly's interesting discovery, published a brief report of his attempt to apply the principle of impaction to impermeable seeds of *Robinia pseudo-Acacia*. A small sample of seed which produced only 15% normal seedlings without treatment when shaken for 20 minutes in a two-liter glass bottle produced 90% normal seedlings in a germination test. A larger quantity which was treated by revolving a steel drum 20 minutes in such a manner that the seeds were thrown from end to end gave approximately 70% normal seedlings. Germination of the treated seed in the field was much greater than untreated. The Forestry Department of Iowa State College then used the mechanical method of impaction on more than a ton of black locust seed with good results in spite of the dry season at the time of planting.

The effect of impaction on impermeable seeds of *Strophostyles helvola, Lespedeza capitata, L. virginica, Amorpha fruticosa, Robinia pseudo-Acacia* and *Glycine max* was investigated (195). With the exception of *S. helvola,* the method gave encouraging results. For example, a sample of seed of *L. capitata* with 4.25% germination and a hard seed content of 78% was shaken ten minutes in a heavy glass bottle. After this treatment the sample contained only 1% hard seeds and gave 83.5% normal sprouts. The same treatment applied to seed of *L. virginica,* which before treatment germinated 3.5% with 75% hard seeds, gave 33% normal seedlings and 34% hard seeds. A sample of seed of *A. fruticosa* before treatment produced 45% normal sprouts and had 25% hard seeds. The ten minute impaction treatment increased the percentage of normal sprouts to 67 and reduced the hard seed content to 0.5% Four lots of *R. pseudo-Acacia* seed were treated with varying but encouraging results. One sample contained 84.8% hard seeds, and a germination test produced 12.8% normal seedlings. Germination in the field gave 6.5% emergence. After impaction this lot produced 82.2% normal seedlings in the laboratory test with 12.8% hard seeds. In the field the emergence was 52%. Two lots of soybeans were treated. One lot, variety Cayuga, contained 74% hard seeds and germinated only 24% in sand. After impaction for ten minutes the sample gave 70% emergence in sand. Further tests were made by the writer in his classes, using seeds of sweet pea (*Lathyrus oderatus* L.), gray sugar pea (*Pisum sativum* L.), *Vicia villosa* and *Glycine usuriensis* Regel & Maack., with results comparable to those with *R. pseudo-Acacia,* soybean and *L. capitata.* The results obtained with these limited attempts to apply the principle of impaction warrant further and more extensive investigations. If properly applied, impaction causes little or no injury to the seeds, since it does not involve the use of acids, other liquids, sand or other abrasive materials. On the other hand, it should not be anticipated that the principle can be applied to all species with impermeable seeds.

The value of impermeable seeds, without treatment, in crop production has received much attention. One of the most extensive studies in recent years was published by Whitcomb who worked with seeds of alfalfa, sweet clover and red clover. He reported that hard seeds of alfalfa germinated 99%, sweet clover 26%, and red clover 39% in the field during one growing season, and further that hard seeds of alfalfa germinated more readily in the field than in the laboratory. Leggatt (246, 247) decided that impermeable alfalfa seeds are equal in value to permeable ones and that 50% of the hard seeds in sweet clover may be considered as germinable under Alberta conditions. Witte, after an extensive international investigation, reported the following percentages of hard seeds as being of value in producing plants in the field: red clover, 34%; alsike, 20%; white clover, 25%; lucern, 72%. Erickson and Porter in tests with 119 different lots of small-seeded legumes concluded that impermeable seeds of alfalfa, red clover and sweet clover germinated in sufficient quantity to contribute to field stand. The largest increases in field stand resulting from impermeable seeds in laboratory samples were 124%, 27% and 112% for alfalfa, red clover and sweet clover, respectively.

In Colorado, Weihing reported that samples of alfalfa seed with less than 20% hard seeds germinated 61% to 64% in the field. Samples with 20% to 62% hard seed gave an average germination of 57% to 60%. He concluded that samples with many hard seeds were practically equal to those with few or no hard seeds in field performance.

These studies of the field value of impermeable seeds have helped seedsmen in their processing and marketing of seeds and have aided farmers in regulating rates of seeding with small-seeded legumes.

Impermeable seeds possess an additional value in that they retain their vitality in dry storage much longer than permeable seeds, and in the soil they enable many plant species to maintain themselves and thus provide useful plant cover as well as forage for livestock. Seeds of species that normally require treatment prior to planting should not be treated until storage because seeds of most species lose their vitality much more rapidly than impermeable seeds.

ADVANCES IN OUR KNOWLEDGE OF DORMANT SEEDS

Determination of the vitality of dormant seeds has long been a difficult problem because of lack of knowledge concerning the various causes of seed dormancy and the methods that could be employed to overcome the dormant condition. Within the past ten years or more, a large number of papers have been published that have supplied information of great value on these two phases. Chief contributors have been workers of the Boyce Thompson Institute for Plant Research at Yonkers, New York, but other individuals have also made valuable contributions. A review of the more pertinent ones is presented herewith.

One of the chief recognized causes of dormancy has been the impervious character of fruit coverings and seed coats in many plant families. Impermeable seeds of the Leguminosae, Malvaceae and Convolvulaceae which have long been considered as possessing a special type of dormancy are not considered here, since they are discussed in the previous section. Closely related to the factor of impermeability is that of unripe and undeveloped embryos; in fact, the two factors often are found in the same plant species, which fact justifies their consideration together.

Dormancy in seeds of many species of Rosaceae has been shown to be caused usually by a combination of impermeable structures and unripe embryos (83). Removal of the stony pericarp from peach seeds increased the rate and percentage of germination which normally requires three to five months at 10° C. Following such removal the embryos were able to after-ripen and germinate more rapidly. Removal of the seed coat of peach seeds (136) permitted germination in ten days. Seeds of apple (*Pyrus* spp.), *P. arbutifolia* Lif., *P. baccata* L., *Amelanchier canadensis* (L.) Medie. and several species of *Rosa* all require after-ripening of the embryos (83) by stratification at temperatures ranging from 1° to 10° C. for varying periods of time. Apple seeds held in dry storage for two and a half years required after-ripening as well as freshly harvested seeds. Removal of the carpels from seeds of 12 or more species of *Crataegus* (137) overcame the seed coat effect and hastened after-ripening. Treatment of the seeds with H_2SO_4 was equivalent to removal of the carpels. After-ripening is also required in cherry seeds (71).

The primary cause of dormancy in seeds of *Tilia* was considered to be the impermeability of the testa and not of the pericarp (429), although Barton (21) considered that in *Tilia americana* L. and several other species the embryo is partially dormant. She was able to shorten the period of germination by four months if the seeds were soaked in H_2SO_4. Spaeth used the same method, although he found that if the embryos were excised or the tips of the hypocotyls freed, then germination occurred promptly. The cause of dormancy (149) in seeds of seven species of *Cotoneaster* was found to be the seed coat factor and unripened embryos. Treatment with H_2SO_4 was effective in overcoming the inhibitory seed coat factor in two species. Germination of the seeds of *Symphoricarpus racemosus* Michx. (135) cannot occur until the inhibiting effect of the seed coat is overcome and the embryo is completely developed and after-ripened. The seed coat can be made permeable by treatment with H_2SO_4 or by fungus invasion (343) which occurs in a moist medium. Normal seeds placed at 25° C. for four months in the absence of fungi failed to germinate after being placed at 5° C. for the after-ripening process. A similar condition was found to exist with seeds of *S. orbiculatus* Moench. (140). Passage through the digestive tract of farm animals (291) hastened germination of some seeds. Seeds of *Polygonum* spp. and *Rumex* spp. after-ripened more rapidly if the pericarp was removed either by hand or with H_2SO_4 (374). The pericarp on fruits of *Polygonum scandens* L. and many other species retarded the rate of gaseous exchange and of water absorption (211). After-ripening of the embryos was hastened by exposure to low temperature and a moist environment. Dry storage failed to bring about complete after-ripening of most species that were studied. Justice (212) considered that the after-ripening process was primarily physiological. The fruits of *Oryzopsis hymenoides* Ricker have an indurated lemma and palea which prevented water absorption, but treatment of the seeds (fruits) with dilute or concentrated H_2SO_4 was found to hasten germination by removing the obstacle created by the glumes (440). Toole (461) found that some of the embryos required an after-ripening period before germination could occur.

In a study of storage conditions affecting the vitality of lettuce seed, Griffiths found that seeds of the variety "New York" were more resistant to high temperature and high humidity than seeds of the variety "Grand Rapids", a condition which he thought was caused by highly suberized membrane surrounding the seed embryo of the former variety. Seeds of *Impatiens balsamina* L., dormant when freshly harvested, required an after-ripening period at 5° C. in a moist medium (234). Pre-chilling was of no value if the seeds were stored dry for two to three months and in some cases caused injury. Removal of seed coats of fresh cucumber seed (479), avocado (128) and tung tree seed (414) hastened the germination of each and resulted in more uniform germination, indicating that the seed coats constituted an obstruction to the entrance of water or to an adequate exchange of gases. Treatment of depulped and dried seed of *Juniperus virginiana* L. for 20 minutes in sodium-lye solution gave quicker germination than occurred from untreated seeds (480). Afanasiev (4) gathered seeds of *Nandina domestica* Thunb. when the fruits had acquired a ripe appearance and found that the embryos were in a rudimentary condition in which the cells were not clearly differentiated. He was unable to overcome, by stratification, forcing agents or changes in time of planting, the natural tendency to germinate only during late fall or early winter. Seeds held in cold storage for nine or ten months kept well with no appreciable loss in vitality. Evidence was obtained by Johnson (204) that delayed germination in seeds of selections of *Avena fatua* is determined by a condition of the seed coat which develops after fertilization. After-ripening was necessary but was found to be longer for the secondary than for the primary grains. Incompletely after-ripened grains, if placed under germinative conditions, developed secondary dormancy. Low, dry storage temperatures retarded after-ripening, but storage in a frozen condition hastened germination. Breaking the seed coat over the embryo or increasing the oxygen concentration around the seeds stimulated germination. Toole and Coffman (460) found that seeds of *A. fatua* exhibited considerable variation in dormancy and that dormancy was not associated with morphologic characters.

As indicated in the preceding review, an understanding of the role of seed coats or fruit coverings in relation to seed dormancy is a preliminary to the determination of seed viability. In some cases the condition of the seed or fruit covering is known to be the primary cause of inability to germinate promptly, but more frequently it appears that after-ripening of the embryo is necessary also.

Treatment or removal of the impervious structures only hastens germination, presumably by providing an earlier opportunity for the embryo to become affected by environmental conditions.

Certain seeds have exhibited a type of dormancy that is related to the exchange of gases in the embryo. Davis (105) reported that the embryos of fruits of *Ambrosia trifida* which are dormant at maturity can be induced to germinate by storage in a saturated condition at 0° to 10° C., and that if the enveloping membrane of after-ripened embryos restricted gaseous exchange sufficiently at temperatures favorable for germination, secondary dormancy developed. This same investigator (104) found that the embryos of seeds of *Xanthium* become dormant when gaseous exchange is restricted. High temperatures induced and low temperatures overcame dormancy. Thornton (45) found that the imbibed upper seeds of cocklebur could be forced into germination by confining the seed in an atmosphere containing 80% oxygen or in mixtures of oxygen and carbon dioxide. Germination of the lower seeds was hastened by addition of carbon dioxide. Embryos of both upper and lower seeds became dormant by keeping them in atmospheres lacking oxygen but composed of N_2, H_2 and CO_2 or mixtures of CO_2 and N_2, for four to 16 weeks at 28° to 31° C. Less oxygen was required for the germination of dormant seeds as the temperature was increased. The same author (455) worked with freshly harvested lettuce seed which is often dormant. Dormancy was overcome by placing the seeds either in darkness or light at 35° C. with the addition of 40% to 80% of CO_2. At lower temperatures less CO_2 was required. These results are of interest because of the earlier work of several investigators which indicated that low temperature, light and a moist substrate are essential to breaking dormancy in lettuce seed. Seeds made dormant by high temperature in the absence of CO_2 germinated after exposure to 40% to 80% CO_2 and 20% O_2 for 96 hours. Thornton, in a more recent paper (457), advanced the idea that secondary dormancy and even primary dormancy may have its inception in the accumulation of intermediate products that are formed by partial anaerobic respiration. These products act as inhibitors because an insufficient supply of oxygen temporarily impairs the oxidation system which is unable to function normally in the removal of harmful products. Seed dormancy may be of long or short duration, depending upon the structure of the seed coat, and the external factors which alter the amount of oxygen available to the embryonic tissues. Popstov, in his study of the seeds of *Taraxacum megalorhizon* Hand.-Mazz., found that secondary dormancy developed if the seeds were exposed to a high (30° C.) or low (0° or 1° C.) temperature, provided that germination had not already started. The optimum temperature for germination of this species was found to be 15° to 20° C., and if freshly harvested seeds were placed at 25° to 30° C., 95% to 98% developed a deep secondary dormancy.

Dormancy in seeds of ash (*Fraxinus*) was studied by Steinbauer (436) who found that the embryos of black ash seeds must undergo considerable enlargement before germination can occur. The temperature most favorable to embryo enlargement was not conducive to seed germination, and mechanical resistance of the enveloping tissue and coats retarded growth of the embryo when the temperature was unfavorable for germination. Embryo enlargement occurred at 20° C., and stratification at 5° C. enabled the fully enlarged embryos to absorb and accumulate reserve materials from the endosperm, after which germination occurred at higher temperatures. Embryos of red, green and white ash were found to be fully enlarged at maturity, and stratification at 5° C. brought about changes favorable to germination.

Several instances of unusual types of dormancy have been reported recently. In some seeds there is a peculiar type of epicotyl dormancy which is characterized by emergence of the root at normal or room temperature, but the stem sprout will not develop until the partially germinated seeds are exposed to a moist low temperature. This condition has been noted in seeds of *Convallaria majalis* L. (36), *Smilacina racemosa* (L.) Dest. (36) and a number of others (31), some of which exhibit both epicotyl and root dormancy. Seeds of four native grasses were observed to possess prolonged dormancy (79) which persisted for 18 months after harvest. Cold or room storage did not break the dormancy. Viability continued for several months after termination of the dormant period. The seeds of *Benzoin aestivale* (L.) Nees (407) required exposure to a warm temperature prior to a low temperature in order to bring about after-ripening. Iris seeds have been reported not only to be dormant at maturity but to require one to three years for normal germination (373). An inhibiting substance has been found in the endosperm adjacent to the embryo which if removed by chipping and leaching in water will permit prompt germination. Dormancy in seeds of *Scirpus* spp. was broken by high illumination and high temperature. Stratification for three to six months broke the dormancy of small-seeded species but not of the large ones. The cause of dormancy was considered to be a physiological condition of the embryo (200).

The relation of stage of seed maturity to dormancy has been studied by several workers. Deming and Robertson tested seeds of several varieties of barley, oats and wheat when they were mostly in the hard dough stage, by planting in moist sand. They found considerable variation among varieties in that some germinated at once, others in 10 to 20 days. Seeds of black winter emmer, bearded winter spelt and einkorn were slightly dormant. Seeds of two varieties of oats germinated in the shock, whereas Kanota wheat seed possessed a long dormant period and resisted sprouting in the shock. Thompson (450), in a study of seed of three varieties of lettuce, obtained evidence that dormancy and light-sensitivity is in part due to immaturity at the time of harvest. Immature seed gave a marked response to light exposure, but mature seed gave little response. He suggested that plant nutrition affects the number of flowers and seeds produced by individual plants which in turn is correlated with dormancy. Sprague (431) made periodic samplings of immature seed corn and found that normal germination did not occur until the moisture content had been reduced to 25% or less. Frosted corn with a high moisture content (362) may not be injured unless the temperature is low, but maximum viability or injury cannot be determined until the seed undergoes marked reduction in moisture. A similar condition (390) was found to exist in seeds of some varieties of sorghum and in Peking soybean. In several varieties of sorghum, dormancy disappeared sooner in unthreshed than in threshed seed. Timothy seed, according to Toole (458), when freshly harvested was partially dormant, and after-ripening occurred more rapidly in cut heads than in threshed seed. Seed of *Poa pratensis* (40) was found to pass through at least two stages of partial dormancy after collection of the heads by hand. Before the seeds were completely air-dry they germinated poorly, became germinable when air-dry and later became partially dormant. Seed of Buffalo grass (301) exhibited varying degrees of dormancy but was less dormant in the hard dough or earlier stage than when mature. Treatment of mature seed burs with H_2SO_4 hastened germination.

Hills, (184–186) investigated dormancy in seeds of subterranean clover and reported that, although an increase in the percentage of seed germination followed a rest period after harvest, the temperature at which the seed was placed for germination was also important, the optimum being 10° C. He found that delayed germination was a varietal character.

The phenomenon of light-sensitivity in seeds of certain species has been observed and recorded for many years. In general, light has been employed as one means of breaking seed dormancy. In some cases its effects have been similar to those of exposure to low temperature or of treatment with potassium nitrate. On the other hand, there is some evidence that the effect of light on some seeds is specific and different from that of other factors. Some seeds germinate poorly or not at all in the dark and vice versa.

Freshly harvested seed of *Mimulus ringens* L. (194) was found to be light-sensitive with a viability of nearly 100%. These properties persisted for several months in dry storage, but the only way that germination could be brought about was to expose the seeds to artificial light for at least one hour at not less than 1.5 foot candles each day, or to full daylight for more than three days. Attempts to effect germination by after-ripening the seeds at low temperature in the dark, or away from sunlight at high temperature by submerging in water, or by rupturing the seed coats, were unsuccessful. Axentjev studied seeds that were known to be inhibited or stimulated by light. Among those that were inhibited he found that the integrity of the seed coat was essential in four species. Among the light-stimulated group the light effect was entirely due to the integrity of the seed coat in two species and of little importance in two other species. Seeds with broken seed coats of four species representing both groups germinated better in both light and dark locations. Increased oxygen pressure increased germination of seeds with uninjured seed coats and vice versa. This author concluded that seed or fruit coat structures restrict the intake of oxygen and that light interferes with or favors oxidation processes within the seeds, according to the species. The favorable effect on germination of new-crop lettuce seed which was found to be in a physiologically unstable condition by Shuck (416) was considered to be caused by the action of light on wet seed. This same author (418) found that light-sensitive lettuce seeds became germinable when placed in a saturated atmosphere and exposed to daylight, a condition which continued if the seeds were dried in the dark or in green light. Daylight and red or blue rays caused reversion to dormancy. Light was required for the germination of 19 samples of tobacco seed (226), but the amount of light required was conditioned by the temperature and the length of time the seeds were soaked. Air-dry seeds were insensitive to light, but the effect of light on imbibed seed was not reversed by subsequent drying. Exposure of imbibed seed to direct sunlight for 0.01 second or to green light induced an increase in germination. Exposure of imbibed seeds to moonlight for 15 minutes induced a high percentage of germination. An alternation of temperatures in the dark gave good germination of seeds that germinate poorly at a constant temperature in the dark. The effect of monochromatic light on light-favored and light-inhibited seed, as determined by Meischke, showed that wave length and germination were not correlated, but each part of the spectrum had a very specific effect, either inhibiting or favoring germination. Antagonistic effects on germination were noted when red and green filters were used in combination. Seeds of three species of *Agrostis* were found by Leggatt (254) to be highly light-sensitive but low light-requiring. Germination capacity was proportional, but germination speed was inversely proportional to light intensity. A seasonal effect was noted in that germination capacity decreased but germination speed increased during February and March. The effect of light was also found to be physiologically different from that of potassium nitrate solution.

Inheritance of dormancy has been studied in seeds of oat (*Avena*) and peanut (*Arachis*). Reciprocal crosses were made (205) between *Avena fatua*, the seeds of which are dormant at maturity, and *A. sativa*, the seeds of which are germinable shortly after harvesting. Germinability was genetically dominant over dormancy. Freshly harvested seed (73) of the varieties of *A. byzantina* Koch., *A. fatua* and *A. sterilis* L. showed slow or delayed germination, whereas seed of varieties of *A. sativa* L. exhibited prompt, slow and delayed germination. Seed of one variety of *A. nuda* L. germinated promptly. The possibility of using seed dormancy to trace the probable origin of varieties was suggested. With the exception of Fulghum, Victoria and *A. sterilis* var. *ludoviciana,* all seeds germinated promptly after storage for seven to ten weeks. Seed of the variety Nortex required at least 66 days for dormancy to disappear, which explained the poor stands that often result when freshly-harvested seed of Red Rustproof strains is planted. Seed of some varieties and species of peanut are dormant at maturity (192) and require from a few weeks to two years before the rest period is over. The mean time required for emergence after planting varied from nine to 50 days for strains of Spanish and Valencia to 110 to 210 days among varieties of *Arachis nambyquarae* Hoehne and *A. Rasteiro* A. Chev. The mean time required for emergence of seedlings from F_1 seed was intermediate, probably slightly greater than the parental mean. Rest period behaviour suggested and agreed closely with the theory of multigenic inheritance.

From the practical standpoint in seed laboratory practice it is more important to know when to expect dormancy in seed samples and how to germinate dormant seeds than to know the cause of dormancy. Several short papers that deal with different kinds of seeds are of interest and value because they deal with methods for germinating dormant seeds. Varieties of Russian winter and spring wheats (60) required a rest period of one to two months after harvest before maximum germination at 23° to 24° C. occurred. The time required for after-ripening served as an index for each variety. At a temperature of 13° to 14° C. seed of all varieties germinated immediately after harvest. Seed of *Pinus strobus* L. (229) germinated promptly at 6° to 12° C. Dormancy in cotton seed (421) was overcome by drying and storing the seed for a short period. Seeds of domestic ryegrass which were found to be dormant when freshly harvested (355) germinated best at a constant temperature of 10° C., but after dormancy was overcome this temperature was slightly injurious. Seeds of *Poa* spp. and of *Dactylis glomerata* (432) were partially dormant when harvested. Pre-chilling at 5° to 10° C. or exposures at 10° to 30° C. or 15° to 30° C. were effective in producing maximum germination. Freshly harvested seed of *Poa pratensis* (8) germinated well at 15° to 30° C. daily temperature with light at the higher temperature and n/100 KNO_3 solution on the seeds. Freshly harvested seed of *Axonopus compressus* Beauv. (9) germinated promptly at daily alterations of 15° to 35° C. plus light and n/50 KNO_3 solution. A temperature of 3° C. for 28 days (462) was required to bring about germination of the seed of *Panicum obtusum,* and four to eight weeks in moist storage at 5° C. (146) was found to be the most effective method of inducing germination of the seed of *P. anceps* Michx. Seeds of *Agrostis tenuis* Sibth. (two varieties) (10), which often exhibit a dormant condition, germinated best when sown in petri dishes on blotters moistened with either 0.1% or 0.2% potassium nitrate solution and placed for 28 days at 10° C. and 30° C. daily alternating temperatures with fluorescent lights (100 foot candles) at the higher temperature. Pre-chilling the seed of belladonna (172) broke dormancy, and germination occurred at alternations of 20° to 30°C., 15° to 30° C. or 35° to 15° C. with 0.2% KNO_3 solution. A bath in dilute uranyl nitrate (385) brought about prompt germination of the seed of *Aesculus hippocastanum* L. which normally requires a rest period. Germination of the seed of Russian dandelion, *Taraxacum koksaghyz* Rod. (259), was hastened by permitting imbibition of water, insufficient to induce germination, followed by immediate drying of the seed. Seed of the Vicland oat variety showed only 40% to 60% germination one or two months after harvest (409). Delayed germination was overcome by February of the following winter. The use of 0.2% KNO_3 solution at 22° C. or chilling for four days at 4° C. gave prompt germination. Seeds of the oat varieties Boone and Tama (40) were found to be similar to Vicland, although in farm storage it appeared that dormancy might be overcome by November or December, then recur in February. Freshly harvested seed of winter barley and wheat (295) germinated as well at 15° C. as when chilled and placed at 20° C. These results are similar to those obtained with Russian wheats.

A recent study of guayule seed (41) showed that the embryo is surrounded by an impermeable seed coat which must be punctured or weakened before the seed will germinate. A standard treatment which effected germination was to wash the seed in water for 12 to 18 hours, using three or four changes of water and then to soak the seed for two hours in sodium hypochlorite solution containing 1.5% of available chlorine at the rate of four ml. per 100 seeds. Other agents, such as hydrogen peroxide, perchloric acid and nitric acid, were as effective as sodium hypochlorite, suggesting that the action of the chemicals on the seed coat is one of oxidation which changes the seed coat structure so that germination may occur.

It is evident from this review of the investigations with dormant seeds that the factors which determine dormancy are very complicated, some being mainly physical, others physiological or a combination of both, but the knowledge now available makes it possible for seed technologists, nurserymen, seedmen and farmers to handle dormant seeds with more intelligence than heretofore. Excision of embryos as a means of determining viability of some kinds of seeds has not received attention in this section but will be discussed in the section that deals with rapid methods for measuring seed viability.

DEVELOPMENT OF METHODS THAT PROLONG SEED VIABILITY

Retention of seed viability is of practical importance to the plant breeder, the seed merchant, the nurseryman and the farmer. The various factors that affect seed longevity have received serious consideration the past decade or more with the result that marked progress has been made in the development of seed storage methods which prolong seed viability. The most important of the factors are stage of maturity when harvested, viability, kind of seed (including genetic factors), moisture content of the seed when stored, air temperature and humidity in the storage room, rate of water absorption or loss by the seed in storage, respiration rate at different temperatures, and atmosphere in which the seed is stored. Any one or a combination or two or more of these factors may be of primary importance to the longevity of a given kind of seed.

In a study of the viability and longevity of seeds of western range and pasture grasses, McAlister harvested seeds of three species each of *Agropyron* and *Bromus*, and one each of *Elymus* and *Stipa* in the pre-milk, milk, dough and mature stages of development and stored them for 58 months. Seeds in the dough stage had viability similar to mature seeds in all species in laboratory tests, but seeds in the pre-milk and milk stages were inferior. In field emergence tests mature seeds were superior to all other types. Nearly complete loss of viability in 58 months occurred in seeds of *Elymus glaucus* Buckley, 18% in *Bromus inermis,* 30% in *Agropyron smithii* Rydb, and 74% in *A. trachycaulum.* Other species retained vitality almost completely. Time of harvesting of seed of velvet bean had a marked effect on viability (216). Likewise seed from upright and vigorous plants possessed higher vitality than seed from lodged or stunted and weak plants. Fully ripe seeds of *Ulmus montana* With. (397) germinated more rapidly than immature seeds, were less susceptible to changes in the storage environment and were superior for nursery plantings. Sound, mature flax seed (109) maintained a vitality of 90% for ten years in good storage, but moist or weather-damaged seed deteriorated rapidly. From a study of the effect of environmental and genetic factors on seed viability and germination, Crescini concluded that self-fertilization, even among pure lines, may result in lines of different germinating power, whereas cross-fertilization may eliminate the lethal factors that are responsible for low germinating power. He also concluded that, in general, percentage viability and readiness to germinate are due to independent genetic factors. Longevity of hemp seeds was considered to be neither hereditary nor correlated with its viability or readiness to germinate.

Seed moisture, air temperature and humidity and atmospheric content, which constitute the storage environment, are interrelated so intimately in their effects on seed longevity that a separate discussion of each is impossible without unnecessary repetition of references. On the other hand, the literature deals with so many different kinds of seeds and so many types of experiments that a brief review is difficult. It has seemed desirable to classify the results of the many investigations, in part at least, in terms of kinds of plants, namely, field crops and grasses, horticultural and floral plants, trees and shrubs.

Seeds of Field Crops and Grasses. Seed longevity of corn, small grains, soybean, sorghum and common grasses stored in rooms where temperature and humidity are influenced by the surrounding air has been recorded by several investigators. Seeds were stored in paper bags in Ireland at room temperature (236). A small but gradual decrease in germination occurred the first few years, followed by rapid deterioration until all were dead. The number of years required for complete loss in vitality was nine for oats, meadow fescue and timothy, ten for meadow foxtail, perennial rye-grass, orchard grass and swede, 13 for Italian ryegrass and flax, and 14 for white clover. In New South Wales seeds of five native grasses harvested in 1925 or 1926 were stored in packets at room temperature and tested each year until 1938 (312). Germination increased in seeds of four species until 1930, then declined. Some seeds were viable in three species until 1935 and in one until 1938. Viability of seed corn (121) was retained well through five years of storage but yielding power declined gradually after the second year. Seeds of corn, cereals, sorghum and soybean were stored in sacks in a dry unheated room for 22 years in Colorado (392) where air humidity is not high. Yellow dent corn germinated well for the first four years but decreased 13% in the fifth year. Rosen rye and Wisconsin black soybean decreased 10% in five years. Amber sorghum germinated well for six years, and seeds of barley, oats and wheat decreased from 7% to 14% in viability the first ten years. Seeds of Atlas, Kafir and other sorghums (501) stored in small bags showed no loss in germination from December to June, but loss in vitality in commercial seed lots was considered to be caused by too much moisture in the seed when stored. Soybean seed three years old stored in a laboratory in Canada (241) germinated satisfactorily, but four year old seed was inferior. The few plants obtained in the field from five to six year old seed were weak and emerged slowly. Cotton seed delinted with H_2SO_4 (163) deteriorated in vitality more in one year of storage than undelinted seed. Satisfactory storage of seed of garden bean, corn, soybean, rice, alfalfa and *Pennisetum ciliare* Link, was obtained in Hawaii (6) by storage in a very dry atmosphere at room temperature, although best results occurred when the temperature was 8° to 10° C. and relative humidity was between 15% and 45%. $CaCl_2$ or CaO in the bottom of an air-tight container maintained a low humidity.

Another factor in the storage environment is that of seed treatment. Cotton seed (285) treated with Ceresan and New Improved Ceresan and stored for one to 17 months in a laboratory and in a ventilated corn crib showed no significant differences in emergence or yield as a result of either period or type of storage, but treated seed gave 25% increase in emergence over untreated seed. Seed corn (231) with 12% moisture treated with mercurial disinfectants at the rate one and a half ounces per bushel and stored under good conditions retained its yielding power for one year.

Seeds of various field crops stored under a controlled environment and tested for viability have yielded valuable data. Seed of several varieties of unhulled peanuts (39) gave high germination when stored at 0° to 5° C. or at 21.1° C. in galvanized iron cans placed in a dry room. Vitality was retained for three to five years, depending on the variety. Chewing's fescue seed (220) lost no vitality in 24 months with moisture contents ranging from 8% to 14% when stored at temperatures of 2° and –10° C. There was a gradual decrease in the period that viability was retained when the storage temperature was changed from 10° to 30° C. and the moisture content of the seed ranged from 10% to 14%, except that seed with 10% moisture lost no vitality in 15 to 24 months at a temperature of 20° C. Cotton seed (422) with a moisture content below 8% and stored in tin containers that prevented reabsorption of moisture retained its vitality well for seven years. Seed with 14% moisture stored at 32.2° C. lost its vitality in four months. Cotton seed stored in bags under natural humid conditions in Charleston, South Carolina, deteriorated rapidly in two years. In Trinidad (344) optimum conditions for storage of cotton seed were found to be a relative humidity of 30% and temperatures between 24° and 26° C. Intensive drying reduced the germinative power, but conditioning of such seed at a relative humidity of 50% for six months increased the percentage of germination over unconditioned seeds which indicated that rapid absorption of moisture by very dry seeds is injurious. Seed corn (231) stored five months with 15% and 17% moisture produced a lower yield than seed with 12% moisture. Cigar wrapper tobacco seed harvested in Florida (227) was placed in ordinary containers, in sealed containers over $CaCl_2$ and in rubber-stopped vials and stored in a basement, an attic, a laboratory and in a refrigerator at 5° C. All seed stored over $CaCl_2$ and in various ways in the refrigerator retained vitality well for 11 years. Seed with only 5.3% moisture in rubber-stopped vials showed a small percentage of viability after eight years of storage.

Seeds of Horticultural and Floral Plants. A considerable body of knowledge relative to the storage of vegetable and floral crop seeds has been published since 1933. Most of the investigators have used controlled environment and seed samples with known percentages of moisture in their experiments.

Onion seed, which is highly sensitive to storage conditions, has received more attention than any other kind, although lettuce, carrot, pepper, eggplant, tomato and several others have been studied. Seeds of lettuce, onion, carrot and pepper (23) were injured in sealed storage at room temperature unless one-third to one-half of the moisture was removed prior to sealing, but at –5° C. drying was not necessary. Sealed storage of undried seed was superior to open storage. Seeds of eggplant and tomato kept well for three years in either sealed or open storage at room temperature. Onion seed with 6% to 8% moisture (38), stored in sealed containers, showed no significant loss in vitality after nine years, and even at 30° C. seed with 6% moisture kept well. A combination of high moisture and high temperature was the most severe treatment. Storage at 5° C. in sealed containers with only 6% moisture was recommended as safest. At relative humidity of 50% or less (24), pepper and other vegetable seeds with high germinating power kept well for three months at 35° C., but at 20° C. or lower the vitality was preserved for a much longer time. Adjustment of moisture content to between 6% and 8% in seeds of onion, lettuce and cauliflower, sealing in air tight containers and storage at low temperature maintained vitality for more than four years. Determination of moisture content in seeds of ten vegetables stored at 26.7° C. and in a relative humidity of 75% to 85% (48) was found to be an unsafe criterion for measuring changes in seed moisture because of loss in dry matter and accumulation of water respiration. The moisture content that will develop at temperature and humidity ranges of 10° to 26.7° C. and 45% to 85% relative humidity was found to follow a linear regression. Wide differences were found among the kinds of seed. Seeds of onion, spinach, sweet corn and peanut, stored at 80% humidity and 26.7° C. deteriorated below good commercial grade in 10 to 20 days. Seeds of beet were injured in 110 days under the same conditions. A humidity between 45% and 50% was found to be a safe level for most kinds of seed. At low temperatures seeds developed dangerously high moisture content in moist storage and were quickly injured if removed to a higher temperature unless they were dried. Onion seed stored in cloth bags or in an unheated room in Texas (51) with a temperature range of 5° to 35° C. germinated only 71% after 20 months, whereas the same seed in cold storage was equally viable after 44 months. Seed in sealed jars under vacuum at low temperature produced better plants than any other seed. In Australia (313) onion seed maintained high vitality only one year under natural conditions but germinated well after two years at 5° to 10° C. A relative humidity of 76% was found to be critical (29) for the storage of onion and dandelion seed, in that the length of time these seeds were kept at said humidity was found to be directly related to the deterioration rate, regardless of the storage conditions before and after the initial exposures.

Griffiths made a critical study of longevity of lettuce seed and found that the most critical factor is moisture content of the seed. His experiments revealed that an increase in moisture from 5% to 10% caused a greater decline in viability than an increase in storage temperature from 20° to 40° C. Within limits the moisture content of the seed varied with the atmospheric humidity, yet at a temperature of 40° C. it was possible to increase the humidity to 50% for 32 weeks of storage without impairing vitality. The critical moisture content of the lettuce seeds used in the experiments was between 3% and 5%. To maintain viability of lettuce seed for three years in sealed containers, it was necessary to dry the seed to one-half the ordinary air-dry water content.

Barton (20, 26) investigated the effect of storage on the longevity of seeds of *Taraxacum, Callistephus, Verbena, Lathyrus, Viola, Venidum, Lilium* and *Delphinium.* She found that at room temperature or at 5° C., sealed storage was superior to open storage regardless of moisture content. If the containers were opened before the end of the storage period the vitality declined more rapidly than otherwise unless the initial moisture content was reduced from 5% to 3.9% in dandelion seed. In a later study (35) this same investigator with Garman found that plants from nine-year-old aster seeds flowered earlier than plants from fresh seed, and lettuce plants grown from 13-year old seed produced heavier heads than plants from fresh seed. Tomato seed with low-vitality after 13 years of common storage produced inferior plants, but similar seed stored at –5° C. produced plants that were equal in every respect to those from fresh seeds. They concluded that storage condition rather than age of seed determined the vigor of the plants that were produced.

Seeds of Trees and Shrubs. Longevity of tree seeds is of special interest to nurserymen and farmers as well as to seed technologists who are requested to test or to supply information about them. Baldwin's recent book (18) is of interest and value in connection with seed storage. Most of the recent contributions have dealt with seeds of coniferous trees, but a few have been concerned with deciduous types including fruit trees and shrubs.

Seeds of *Pyrus* spp. (Patten, Greening and Wealthy) and *P. baccata* hybrid which had been in storage for two and one-half years (83) produced a perfect stand of vigorous seedlings after proper after-ripening, and seeds of several species of *Rosa* lost relatively little in germinability after two to four years in dry storage. Seeds of mulberry (444) stored over H_2SO_4 or $CaCl_2$ maintained a moisture percentage of 4.1 to 4.2 and gave 90% germination after seven years. Seeds in ordinary storage with 7% to 10% moisture retained high germinability for only four months. Seeds of *Populus* spp. in Alberta, Canada, live under natural conditions only two to four weeks. Seeds of *P. tremuloides* Michx. (289) stored in open dishes germinated 100% at the end of four weeks, but only 45% after eight weeks. As the relative humidity of the storage atmosphere increased beyond the optimum of 10% a marked reduction in germinability occurred. Seeds of the Russian poplar (*P. Petrowskyana*) Schrod. when kept over $CaCl_2$ at –5° C. germinated 70% after two years. Seeds of elm (25) were injured if stored in an atmosphere of oxygen when the moisture content and storage temperature were high. Vitality was retained for 16 months by sealed storage at 5° C. with a moisture content of 7% in the seeds. Seeds of *Fraxinus pennsylvanica* Marsh. and *F. excelsior* L. (33) with moisture contents of 7% and 10%, respectively, were germinable after seven years in sealed storage at 5° C.

Several investigators have published results of tests with seeds of coniferous trees which included species of *Abies, Larix, Picea, Pinus* and *Tsuga.* Seeds of *Abies nobilis* Lindley retained its germinability for five years when held at –10° C. (199), and larch and spruce seeds stored for three and five years, respectively, in the cones in a dry, cool, well ventilated place retained their vitality well (474). Seed of sugar pine (286) declined from 89% to 28% in vitality after storage for six years at 40° C. In general, the data on storage and longevity of coniferous seeds (22, 175, 322, 394) have shown that sealed or unsealed storage of seeds with low moisture content (5% to 8%) at temperatures from -5° to 5° C. is the most effective environment for the maintenance of viability. Longevity varied from four to ten years, depending upon the species.

Much of the data on seed storage has indicated that a moisture content considerably below that of the air-dry condition together with a low storage temperature is essential to seed longevity. For many species sealed storage is also required. In contrast, there are a few species that require moist storage. For example, coffee seeds were found to keep best for planting during the rainy season in the Dutch East Indies (470) by drying to a moisture content of 40% and storing them in jute bags with a layer of moistened charcoal surrounding the bags. Remoistening of the charcoal every month was necessary. In this condition the viability was 94% and 78% after six and nine months, respectively. Seeds of grapefruit and sweet orange (3) remained viable longest in open storage in a humid atmosphere at 5° C. where the moisture content of the seeds was reduced to only 17%. The seeds of *Montezuma speciosissima.* Moc. & Sess. (32), which declined significantly in vitality within two weeks at ordinary storage, remained viable for four weeks at 5° C. when the moisture content was 33% of the wet weight. Drying the seeds to 10% moisture reduced the germinability by one-half.

Reabsorption of moisture by seeds in storage has been found to vary greatly with the species. Boswell *et al.* found that among several species of vegetable seeds, peanut seed had the lowest moisture content at a given humidity and changed the least with change of humidity, whereas kidney bean seed attained the highest moisture content at high humidity and changed the most with increase of humidity. Cabbage and tomato seed underwent changes only slightly greater than peanut seed. On the other hand, the deterioration of different kinds of seed kept at high humidity and high temperature was not always in proportion to their relative moisture-absorbing capacity. Barton (28) reached similar conclusions. She found that in the order of increasing water-absorption capacity the seeds were peanut, lettuce, flax, pine, tomato and onion, and that this order persisted regardless of atmospheric humidity or storage temperature. Less water was absorbed at the higher than at the lower temperatures at the same relative humidities, and although there was no direct relation between amount of water absorbed and germinability, seeds with high initial vitality were less affected by changes in storage environment than those with low initial vitality. Moutia reported that seed corn with an initial moisture content of 9% absorbed more water at 70% to 90% relative humidity than at a lower level, whereas seed declined from an initial moisture percentage of 13.3 to 10 and increased to 15 when submitted to relative humidities of 60% to 70% and 90% respectively. Humphries and Hurst stored seeds and hay under controlled temperatures and humidities and concluded that relative humidity governs moisture content more than temperature and that particles freely exposed to the air respond rapidly to sudden changes in relative humidity, whereas those in columns in containers do so slowly.

The effect of gas content in the storage environment on seed longevity is of interest and importance. Seeds of *Glycine soja* Sieb. and Zucc. (161) kept in an atmosphere deprived only of oxygen responded similarly to storage in a vacuum in that longevity was extended. Rice seeds (233) stored in zinc vessels containing only CO_2 or air were germinable after four years (97% to 99%), and those stored in straw in similar containers were non-germinable. Seed corn (404) with 11% moisture stored at 30° C. in sealed glass tubes containing oxygen died in three years, but seeds with 11% to 18% moisture in tubes containing O_2, CO_2, N_2 or air and stored at low temperatures germinated well after five years.

The relation of respiratory activity to seed longevity has long been recognized. Geddes found that the respiratory activity of the germ of cereal grains is several times that of the endosperm, that moisture content of stored grain is a major factor affecting the respiration intensity and that accumulation of CO_2 depresses respiration. He also found that at equivalent moistures the respiratory rate of oil seeds (soybean, flax) is much greater than that of cereal grains. Barton (34) studied the respiratory quotient of *Amaranthus retroflexus* L. seeds in moist storage at a temperature of 20° C. which prevented germination. The gaseous exchange measured for 901 days showed a ten-fold reduction in respiration, and the respiratory quotient was always less than one.

The extensive review of the literature on seed longevity by Crocker in 1938 included data on the germination of seed kept in plant herbaria as well as of buried seeds. Since that time a report has been made (12) of the germination of seeds of *Albizzia julibrissin* Durazz. after storage for about 147 years in the British Museum. Turner reported that seeds of *Anthyllis vulneraria* L. and *Trifolium striatum* L. were viable after 90 years and those of *Lotus uliginosus* Schurch. and *Cytisus scoparius* L. after 81 years. An earlier paper (406) published in 1936 gives data on the longevity of seeds of the Norwegian herbarium at Oslo. The seeds were collected between the years 1820 and 1898, and thus varied in age from 34 to 112 years. A total of 1,254 different batches of seeds with few duplications was available for test. Representatives of the Leguminosae, Malvaceae and Convolvulaceae families showed the most longevity. Seeds of *Astragalus utriger* Pall. 82 years old germinated 6%, those of *Sphaeralcea abutiloides* G. Don. 74 years old germinated 43% and those of *Convolvulus flavus* Willd. 64 years old germinated 15%. Plants grown from seeds of ten species of old seeds produced normal plants with few exceptions. Seeds of *Convolvulus arvensis* (55) from a Belgian herbarium germinated 62% at 50 years of age.

Recent reports of tests with buried seeds include both crop and weed seeds and are of special significance in connection with weed control programs. Tests of 60-year old seeds from the Dr. Beal's buried seed experiment (95) revealed that the seeds of four species were still viable, namely, *Oenothera biennis* L., *Verbascum Blattaria* L., *Rumex crispus* and *Silene noctiflora* L. A rapid decline occurred (55) after burial for three years at six to 18 inches among seeds of *Lepidium draba* L., *L. repens* Boiss., *Hymenophysa pubescens* C. A. Meyer, *Centaurea calcitrapa* L. and *Solanum elaeagnifolium* Cav., but seeds of *Convolvulus arvensis, Euphorbia Esula* L. and *Centaurea repens* L. lost little viability at either depth. Seeds of *Solanum carolinense* L. maintained their vitality well after three years at a depth of six inches in the soil. Seeds of buffalo grass (261) found in the walls of a sod house that had been built about 25 years gave 28% germination. At the end of 39 or 40 years the buried seed experiment initiated by J. W. T. Duvel (459) was terminated, and seeds of 16 species, representing ten plant families were still viable, namely, *Abutilon theophrasti* Medic., *Ambrosia artemisiifolia* L., *Convolvulus sepium* L., *Datura stramonium* L., *Ipomoea lacunosa* L., *Lespedeza intermedia* Nakai, *Nicotiana tabacum* L., *Oenothera biennis* L., *Onopordum acanthium* L., *Phytolacca americana* L., *Potentilla norvegica* L., *Robinia pseudo-Acacia, Rudbeckia hirta* L., *Solanum nigrum* L., *Trifolium pratense* and *Verbascum thapsus* L.

(*Concluded in next issue*)

THE BOTANICAL REVIEW

VOL. XV MAY, 1949 No. 5

RECENT DEVELOPMENTS IN SEED TECHNOLOGY

(Concluded)

R. H. PORTER

American International Association, Brazil

RAPID METHODS OF MEASURING SEED VIABILITY 283
THE RELATIONSHIP OF HORMONES AND INHIBITORS TO SEED GERMINATION 289
RECOGNITION OF THE IMPORTANCE OF SEED-BORNE ORGANISMS IN SEED-LABORATORY PRACTICE 293
  Detection and Importance of Seed-Borne Organisms ... 293
  Lists of Seed-Borne Organisms and Viruses 302
USE OF SEED FUNGICIDES IN SEED TESTING 302
DEVELOPMENT OF LABORATORY METHODS FOR MEASURING SEED GERMINABILITY UNDER ADVERSE CONDITIONS 315
APPLICATION OF STATISTICAL METHODS TO SEED TESTING .. 320
ACKNOWLEDGMENTS 322
LITERATURE CITED 322

RAPID METHODS OF MEASURING SEED VIABILITY

The prolonged dormancy of many kinds of seeds, as well as the long period of time required to complete a germination test of some non-dormant seeds, has naturally interested those who work with seeds in a study of other methods that might be used to quickly determine potential germinability. Furthermore, it is often desirable to know the viability of a seed lot within a few hours; hence the demand for rapid methods has become somewhat general.

The principal short-cut methods that have been investigated are (*a*) measurement of enzymatic activity of seeds, (*b*) excision of embryos and (*c*) immersion of seeds in reagents which differentiate between viable and non-viable seeds by color reaction.

Measurement of catalase activity as an indicator of seed viability represents the major work with seed enzymes. The ratio of the catalase of poor seed to that of viable seed of the same kind was considered to express very closely the percentage germination of the seed. Davis (103) concluded that disappearance of catalase from dead seeds was sometimes slow and was dependent on such factors as temperature, moisture and aeration to which seeds are subjected before and after death. He found, however, that the catalase of dead seeds disorganized very rapidly if they were soaked for a time in warm water at a temperature not high enough to affect their viability, but at the same time the catalase activity of viable seeds frequently increased. Using this method and comparing the catalase activity of soaked and dry seeds, Davis concluded that a quick estimation could be made of the viability of dormant embryos, quickly germinating seeds and those that have a long germination period.

Since 1930 a number of workers have tested the catalase ratio method on seeds of apple, peach and cherry (171), wheat (248), several species of coniferous seeds (17), a number of field crops and leguminous seeds (316, 324, 425), and species of *Quercus* (56). A ratio of one or more has indicated high viability, and less than one has indicated low viability, but at the best it has been possible only to estimate approximate viability. With acorns of *Quercus,* measurements of catalase activity were of no practical value in interpreting either the progress of after-ripening or the germinability of the acorns.

Amylase activity in dry and soaked seeds of soybean and oat samples representing lots with high, medium and low vitality was determined by Mar. He found a high positive correlation between amylase activity and vitality in soaked seed of oats but not in dry seeds, nor in either soaked or dry soybean seed. Soybean seed was found (323) to contain amylase of a different type than occurs in seeds of cereals. Phenolase activity (102) was high in young seeds and low in old seeds with low vitality.

Determination of the relation between enzymatic activity and seed viability is of scientific interest and value, but at best it appears that only a close approximation of seed viability can be obtained. Present seed laws which require labeling in terms of percentage of viable seed preclude the use of estimated or approximate viability except in specific cases.

One of the most practical and successful short-cut methods of determining seed viability is to excise the embryos and observe their behaviour under pre-determined conditions that favor their development. Two types of development of excised peach embryos taken together (466) compared favorably with the germination percentage obtained after 12 weeks of after-ripening at 5° C. The time required for the excision method was only seven days. Naked embryos were sterilized in 2% chlorine solution for 30 minutes and then transferred to sterilized one-half ounce bottles on two-thirds agar medium. Those with cotyledons spread at an angle of 75° and those with shoot and root growth were classed as viable. These results agreed closely with the percent germination from fall plantings under nursery conditions. Flemion (136) obtained similar results with peach seeds by placing the naked embryos in moist granulated peat moss at room temperature for ten days. She later (138) modified the method by using filter paper in petri dishes on which to place excised embryos of a variety of different types of dormant seeds. She kept them at room temperature (21° to 23° C.) and found that non-viable embryos deteriorated rapidly. Her results agreed well with actual germination tests. The method was applicable to seeds of the fringe tree which has an epicotyl dormancy. In a subsequent paper (139) she reported that the embryo excision method had been extended to 24 additional species in 11 families and that it was especially applicable to hard coated seeds or to other types in which excision presents special problems. Presoaking, acid treatment and cracking of the outer coats were useful methods that aided in the excision procedures. Heit and Nelson applied the excision method to seeds with extremely dormant embryos and reported that ten to 14 days represented the average time required to make an approximate germination. Non-viable embryos discolored or decayed, while viable ones swelled, showed greening or spreading of the cotyledons or germinated. In all of the experiments with excised embryos the results were compared with actual germination tests which required various types of after-ripening, and the agreement was reasonably close. The excision method is apparently a reliable one when used by an experienced technician.

Immersion of seeds in solutions that give a color reaction has received considerable attention since 1925. The compounds used have been indigo-carmine, dinitro benzene, selenium and tellurium salts, and tetrazolium chloride.

Neljubow tested several organic dyes which do not penetrate living tissue but readily enter dead tissue. The most promising one was indigo-carmine solution 1:2,000. Several investigators in recent years (235, 345) have used this dye with varying degrees of success. It appears that the relationship between the degree of staining and the viability must be worked out for each species. Shaefer-Safonova *et al.*, in tests with seeds of *Caragana arborescens* Lam., *Pinus silvestris* L. and *Picea excelsa* Link, found that it was necessary to remove the seed coats of *Caragana* and the endosperm in seed of pine and spruce. In *Caragana* seeds the tissue most commonly dead is the radicle, and if the apical cell stained, it indicated that the embryo could not produce a seedling. Since the apical cell is close to the root tip, a stain of more than a dot was taken to indicate a non-viable embryo. In pine and spruce the apical cells of the radicle were found to be one-third the distance from the radicle end of the embryo. Tskhoidze used solutions of indigo-carmine on seeds of the tung-oil tree at dilutions as low as 1:4,000 and reported that a viability test of 100 seeds could be made in two hours. Hao tested seeds of *Pinus, Picea, Pseudotsuga* and *Abies* by soaking the embryos in 0.5% and 1.0% solutions for one hour. Embryos that remained white or that had only a small blue spot at the root end were considered viable. He considered this dye superior to selenium salts because it is quicker and is not poisonous.

Gurewitsch developed the dinitro benzene method which involves immersion of the seeds in an aqueous solution of finely divided dinitro benzene for five hours at 15° to 17° C. or one hour at 42° to 45° C., after which they are placed in an aqueous ammonium solution for ten minutes. Living cells have the ability to reduce the dinitro benzene to nitro-phenylhydroxylamine which yields a purple color with NH_3. Seeds were cut near the epicotyl so that it would appear in the cross-section. The chemical entered the seed coats of wheat and rye readily, but it is poisonous and must be used with care. Viable seeds showed a dark purple-colored epicotyl. Weise confirmed the results of Gurewitsch and used the method on seeds of peas and beans with success but with failure on seeds of asparagus and fruit trees. He considered that dinitro benzene could be used to test viability but not germinative capacity of seeds which require special treatment to induce germination.

Treatment of seeds with salts of selenium and tellurium was used by Eidmann. He observed that living cells of seeds reduced these salts to form free selenium and tellurium, respectively, which could be identified by their color. He preferred selenium salts in spite of their poisonous properties. Hasegawa tested both classes of compounds on seeds of Japanese cedar, cypress and pine, and reported satisfactory results. Natlacen used selenium ($NaHSeO_3$) solution on seven species of conifer and seven of broad leaf trees. He soaked the seeds 24 hours in water, then 28 hours in the selenium solution, and stated that the test gave the germination capacity of seed lots. Others (127, 237, 287, 396, 476), too, have tested selenium salts on different types of seeds. One of them (237) obtained results on wheat, barley, rye and oat seeds that compared favorably with actual germination tests. Wach used a 2% solution of $NaHSeO_3$ in which the seeds of *Pinus, Picea* and *Fagus* were soaked for 48 hours. Living seeds were colored red, but boiled, ground, mashed and finely cut seeds and embryos were not colored. He found that changing environmental conditions influenced the degree of coloring and that entrance of the chemical was facilitated by removal of the seed coats in many species. With conifers he noted that the endosperm was colored only if the seeds had started to germinate, which indicated that coloring occurs only when stored reserve material has formed a diastase. Amorphous selenium was first deposited in the inter-cellular spaces and the cell walls, then inside the cells of pine and beech embryos. He was unable to recommend a standard color scale for all species.

Lakon (238, 239) found the use of selenium salts distasteful because of the unpleasant odor and dangerous because of their poisonous properties. He discovered that two salts of tetrazolium in dilutions of 0.5% to 2% were satisfactory and reliable for the determination of viability of corn, barley, oat, rye and wheat seed. He used 2,3-diphenyl-5-methyl-tetrazolium chloride and 2,3,5-triphenyl-tetrazolium chloride but preferred the latter. His procedure was to soak the seeds for 18 to 24 hours, then cut them in half by a median section through the embryo so that the plumule and radicle were split longitudinally. Immediately after sectioning the halves were placed in the tetrazolium solution in petri dishes and kept in the dark for three to four hours. The living tissues of the embryos stained orange to red color because they reduced the tetrazolium salt. He illustrated by drawings the stained area that indicated viability. He did not consider scutellar staining as absolutely essential in all cases, but in corn embryos if no staining occurred below the region from which secondary roots are produced he considered the embryo as viable and capable of producing a seedling. Absence of the stain in the plumule indicated a non-germinable seed. The results of the staining method agreed reasonably well with actual germination tests.

Using 2,3,5-triphenyl-tetrazolium chloride in solutions of 0.5% to 2%, Porter *et al.* (365) treated seeds of corn, barley, oats, wheat, rice, rye, sorghum, soybean, pea, buckwheat and Bahia grass (*Paspalum notatum*) Flugge. for different periods of time. The treatments were preceded by different periods of presoaking in water at different temperatures. A sample of seed corn with 98% to 100% germinability was tested by this staining method, and 99% to 100% of the sections gave the characteristic orange-red color. When presoaked seeds of this lot were immersed in boiling water for a five minute period and placed in the germinator, none grew, and similar seeds also remained free from stain in the tetrazolium solution. All lots of seed that were studied were tested by regular germination procedures, and the results agreed well with those obtained by the staining method. The results with seed of Bahia grass were of special interest because dehulling is a necessary procedure to insure prompt results. The sectioning and staining procedure was used after the seeds remained two to three days in water at 20° to 35° C. The results were highly significant. The value of the tetrazolium method with seeds of soybean or garden bean was recognized as questionable because of the frequent occurrence of injured plumules.

It appears on the basis of present information that the tetrazolium method is superior to others that employ dyes or reducible salts and that it has considerable practical application for a quick evaluation of certain kinds of seed, especially of the Gramineae.

Other methods that have been used to measure seed viability are plasmolysis, detection of respiration by indicators, and endosperm injury. Doroshenko and Niethammer (325) used a plasmolytic method to measure seed viability. The latter investigator used salts of heavy metals, such as nickel, potassium, copper, zinc and cobalt in concentrations of one-quarter to one molar. After soaking seeds in these solutions long enough to cause plasmolysis he made microscopic sections of the swollen seeds and examined them. He was able to make an approximate estimation of viability by this method. Afanasiev (3) reported that if the endosperm of seed of *Magnolia acuminata* L. is injured and the seed placed under germinative conditions, the viable seeds develop a green pigment.

THE RELATIONSHIP OF HORMONES AND INHIBITORS TO SEED GERMINATION

The discovery of growth-promoting substances has resulted in their extensive use in greenhouses, nurseries and orchards. The effect of these new compounds on the germination of seeds has been investigated, but, in general, their value has been shown to be limited. Simultaneously the existence of substances in seeds which inhibit germination has received attention. A review of the more pertinent literature dealing with stimulants and inhibitors as related to seed germination is presented herewith.

Germination of seeds of some parasitic plants has been known to be dependent on the stimulating effect of substances produced by the host plant. Bartsinskii in 1934 found that sunflower roots contain a substance which stimulates the germination of the seeds of *Orobanche cumana* Wallr., a parasite on sunflower. The substance is thermostabile and water-soluble. An extract of sunflower roots applied to soil infested with the seeds of the parasite caused prompt seed germination, and sunflowers planted after the seedlings had been killed remained free from infection. Seeds of *Striga lutea* Lour. (57), which normally germinate only after exposure to a stimulant produced by one of its hosts, were partially stimulated to germinate by first incubating them on moist filter paper for 21 days at 22° C. and then placing them on filter paper moistened with a solution of allylthiourea. Partial control of parasitic plants by artificially inducing germination of their seeds in soil prior to planting of susceptible host plants thus appears possible.

Seeds of varieties of lettuce when freshly harvested have responded favorably to treatment with various substances. Certain sulfur-bearing compounds in aqueous solutions of 0.5% (453) stimulated the germination of dormant lettuce seed at temperatures above 25° C. Thiourea was the most generally effective, but thiosemicarbazide, thioacetamide, allylthiourea, ammonium thiocyanate and potassium thiocyanate were also stimulatory. Later results with thiourea (452) indicated that the best treatment was to soak the seed in darkness for eight to ten hours at 18° C. in a 0.5% solution and then place them to germinate at temperatures between 25° and 35° C. Seed thus treated germinated 87%, untreated seed only 1%. Dormant seed (145) of the lettuce varieties White Boston, Grand Rapids, Iceberg and Black-seeded Simpson which was soaked in 0.5% and 1.0% solutions of thiourea and germinated immediately, displayed a gradual increase in germination with the lengthening of the period of dry storage between harvest and treatment. The degree of stimulation by thiourea was affected by differences in variety and age of the seed.

Injurious effects resulting from treatment of cereal seeds with formaldehyde, copper sulfate and hot water were largely overcome, according to Grace, by adding either 1-naphthylacetic acid or 3-indolylacetic acid to the disinfecting solutions. The optimum concentration of the hormones for individual varieties of cereals lay between 0.01 and five parts per million. This method may have a practical application, especially for wheat and barley seed treated with hot water to control the fungi that cause loose smuts. Landau reported that hetero-auxin at concentrations of 10^{-7} and 10^{-9} stimulated the germination of seeds of oat, French beans, tomato and radish. Thiourea (43) had no effect on dormant barley seed but suppressed mold growth, the presence of which interferes with an estimation of germinability. Molds and common seed-borne fungi of peach (467) were reduced by treating the seed with thiourea, and seed dormancy of the Lovell variety was overcome by the treatment. Seeds of endive were stimulated to germinate by thiourea at high temperatures (451). Colchicine, which is used extensively as a practical seed treatment for the production of polyploid plants, was found by Bond to stimulate germination of seeds of *Petunia axillaris* BSP. The percentage germination was increased and the germination time reduced by soaking the seeds in different concentrations, the optimum being a 0.04% solution.

Popular interest in the culture of orchids has been stimulated by the discovery of methods that promote seed germination. Mariat used a symbiotic, jellied and sugared Knop solution to which he added 2.6 mgs. of vitamin B_1 per liter of solution. Seeds of *Cattleya* orchids placed in this solution gave 42% of the embryos with leaves within three months, as compared with only 3% when no vitamin was added. Meyer added the juice of vine-ripened tomatoes to solid agar on which seeds of the orchid genera *Laelia, Cattleya, Epidendrum, Enyclia* and *Rodrigiasa* were germinated. Seedling development was greater on this than in the Knudson medium, and pH adjustment was unnecessary if the medium was sterilized the same day the juice was prepared. The formula for preparing the medium consisted of 250 mls. of tomato juice and water each and 7.5 grams of agar sticks.

In contrast to the stimulatory effect of growth-promoting and other substances on seed germination, a number of workers have found little or no beneficial effect and in some cases injury from the use of various substances in certain concentrations. The compounds used with little or no benefit on a great variety of plant seeds include ascorbic acid, colchicine, Hormodin "A", indoleacetic acid, indolebutyric acid, indol-3-acetic acid, K a-naphthalene-acetate, lacto flavin, levulinic acid, naphthalene acetamide, naphthalene acetic acid, thiourea, vitamin B_1 and 13 commercial hormone dusts. The kinds of seeds that were tested included tomato (98), wheat and soybeans (499), oats and broad bean (196), sugar beet (331), rice, barley and corn (341), seeds of many other agricultural crops (108, 230), corn (228), rice, corn, cotton, sorgo and soybean (19), seeds representing field crops, vegetable, grasses and flowers (27), vegetables of many kinds (326, 339), 37 types of herbaceous plants (63) and 41 varieties of woody perennials (443). The treatments were applied as liquids and dusts in various concentrations. The list of treated seeds included both dormant and non-dormant types, and in most cases plantings were made in the field to observe plant growth and yield. In all these experiments significant increases in germination were rare; generally germination was the same or less than from untreated seed.

The data that have been published relative to the effect of growth-promoting and other substances on dormant and non-dormant seeds of many kinds of plants indicate that their value is limited to a very few special types, such as dormant lettuce seed, injured cereal seeds and seeds of orchids and some parasitic plants. Until more data to the contrary are accumulated it appears that general use of these substances is not warranted.

One of the earlier reports of a possible inhibitor to seed germination was made by Sagaguchi in 1930, who found that peach seeds in ripened fruits failed to germinate. Scott *et al.* found that fermented peach juice contains a volatile, ether-extractable substance which can destroy seed viability. Seeds that remained in rotting pulp for ten days or in fermented juice for seven days were incapable of germination. Reinhard reported in 1933 that the juice of ripened tomato fruits contains a substance which strongly inhibits germination of tomato seed and displays a less pronounced effect on lens seeds. Tomato juice was found (338) to inhibit the germination of seeds of sunflower, lettuce, carrot, cabbage and endive as well as tomato. The juice of fruits of *Cucumus sativus* L. and of *Carica papaya* L. had a similar effect. A substance (263) was isolated from the cultures of *Fusarium vasinfectum* Atkinson and *F. lycopersici* which depressed or inhibited the germination of seeds. This substance was found to be one of the primary amino compounds, thermostabile and non-specific. Water-soluble nitrogen compounds in sugar beet seed balls (441), from which ammonia is hydrolized, exhibited an injurious effect in the early stages of germination. Washing or presoaking removed the substance.

Freshly harvested lettuce seed was reported to contain a substance (417) which diffuses into the sub-stratum in which the seeds are germinated. This substance inhibited the germination of seeds placed subsequently in the same substratum. The effect appeared to be one of secondary dormancy. Non-dormant lettuce seed was made dormant (332) by immersing the seed for 24 hours at 18° to 20° C. in 25 parts per million of coumarin solution. The inhibitive effects of coumarin was overcome by the use of methods which break dormancy, namely, exposure to light, pre-chilling and treatment with thiourea.

The presence of an inhibitory substance (373) in the endosperm of freshly-harvested iris seed was found to be responsible for the pronounced dormancy. If the seed embryos were in contact with only a small portion of endosperm the inhibitory effect was evident. The inhibitive substance was removed by leaching the seeds in running water. Similarly the pronounced dormancy in seed of certain strains of cabbage (80) was shown to be caused by the presence of a substance in the seed coats. This substance was present in the water-soluble fraction of alcoholic extracts from the seed coats. Immersion of seed for one minute in H_2SO_4 at 4° C. followed by washing in cold, running water and finally by soaking for several hours before planting removed the inhibiting substance.

In contrast to the presence of inhibitive substances in freshly-harvested seeds, old seeds of *Oenothera berteriana* Spach. with low vitality were found to contain a substance which inhibited the germination of highly viable seeds (408). This phenomenon was demonstrated by treating fresh seeds with extract or mash from old seeds.

RECOGNITION OF THE IMPORTANCE OF SEED-BORNE ORGANISMS IN SEED-LABORATORY PRACTICE

Seed transmission of plant pathogens has been recognized by plant pathologists as an important factor in the dissemination of diseases for at least 75 years. A considerable body of information has been published during that time relative to the manner in which pathogens are carried. Seed laboratories have been established in North America and Europe for 50 to 75 years, yet little attention was given to the pathological condition of seed as one aspect of seed quality until the past 15 years or more. There were at least three important reasons for this lag in the application of pathological techniques to the practice of seed testing, namely, (*a*) uncoordinated effort in agricultural experiment stations between phytopathologists and those responsible for the organization and development of seed laboratories, (*b*) lack of training and experience in the field of phytopathology among seed analysts, in general, and (*c*) inadequate methods of detecting the presence or evaluating the significance of organisms carried by seeds. Since 1930 there have been two important developments, (*a*) an active research program in a few centers which has been concerned primarily with practical methods for determining the presence of seed-borne organisms and their effect on seed germination, and (*b*) compilation of lists of seed-borne parasites together with the discovery of new ones not hitherto reported.

Detection and Importance of Seed-Borne Organisms. Examination of seed lots to determine their pathological condition has received major attention in Europe and North America. Zybina, working in Russia in 1927–28, examined 300 samples of flax seed from all parts of the world for the presence of micro-organisms and published the results in 1929. Infection of seeds varied from 4% to 83%, and in the field the degree of seedling mortality correlated well with seed infection except where soil infestation was a factor. On new land he found that 75% of the infection was caused by *Fusarium lini* Bolley and 37% by *Colletotrichum linicolum* Peth. and Laff. Between 1926 and 1930 Doyer in Holland (115) examined commercial seed lots of flax, peas, beet, beans, wheat and barley. She found that 74% of the flax samples were infected in varying degrees with *Botrytis cinerea* f. *lini* (Pers.) and that after two years the fungus had lost its vitality. In one year 84% of the samples were infected with *C. linicolum*. Other organisms detected on seeds were *Ascochyta pisi* Lib. on pea, *Macrosporium* and bacterial infection of bean, *Phoma betae* Frank on beets, *Fusarium* on wheat and *Helminthosporium sativum* P. K. and B., *H. gramineum* (R.) Erick. and *H. teres* Sacc. on barley. This same author in 1934 (116) listed fungi found on clover seed as *Botrytis cinerea* Pers., *B. trifolii, Fusarium, Macrosporium* spp. *Typhula trifolii* Rostr. and *Sclerotinia trifoliorum* Eriks., *A. pisi* (118) was found in varying degrees up to 70% on a large number of pea seed samples. A useful hand book (117) was issued in 1938 which gives the general classification of seed-borne organisms, methods for their detection and suggestions for their control by cleaning and treating. A list of crop seeds with organisms carried by them is included together with illustrations of their symptoms and morphology.

Jorgensen in Denmark examined 89 seed lots of celery seed from Denmark, Germany and France, and reported that 88 of them carried infection with *Septoria apii.* He also found *Phoma rostrupii* Sacc. on carrot seed from plants artificially sprayed when in flower with conidia of the fungus. Muskett and Malone developed the Ulster method for examining flax seed to determine seed-borne parasites by plating, on 2% malt extract agar, from 100 to 500 seeds of a given lot, placing only ten seeds in each petri dish. After incubation for five days at 22° C. they examined the medium around each seed for parasitic organisms. Neergaard (317) made an extensive survey of the seed-borne fungous flora of horticultural crops. He published a list of a large number of plants with the organisms that may be carried by the seeds together with information as to frequency of seed infection, based largely on his own personal experience with seed testing in Denmark.

Moore examined nearly 30,000 samples of seed between 1925 and 1943 for the presence of seed-borne organisms. About 2% of the samples were rejected because of parasitic organisms and insect pests. Samples of peas, celery and parsley were more commonly rejected than other kinds. Bean seed infected with *Phytomonas phaseolicola* and *Colletotrichum* spp., sunflower seed with *Sclerotium sclerotiorum* (Lib.) Mass. and beet seed with *Uromyces betae* (Pers.) Tul. and *Phoma betae* were also rejected.

Detection of organisms carried by seeds in a North American seed laboratory was undertaken by Munn (292) as early as 1921 when he found spores of *Alternaria cucurbitae* Let. on watermelon seed. Another early attempt to apply phytopathological techniques to seed lots for the detection of seed-borne organisms was initiated in Iowa in 1921 (347). Ear tests of farmers' seed corn in lots of 100 ears from each of more than 100 farmers were used to determine the percentage of ears infected with *Diplodia zeae* (Schw.) Lev. Following the tests by the modified rag-doll method, seed samples of nearly disease-free and of diseased seed were prepared from each lot together with a sample prepared independently by each farmer. All samples were planted with three replications from which stand and yield data were obtained. The results showed that the samples of diseased seed gave the lowest stand and the lowest yield, the nearly disease-free gave the highest, and seed prepared by farmers was intermediate in its behavior. This method of testing seed corn for infection with dry rot fungi was continued and expanded to include work with cereal crops and indexing of onion bulbs suspected of being infected with yellow dwarf virus. Methods of determining smuts of cereal crop seeds and organisms on vegetable crop seeds were described. The results were presented at the Annual Meetings of the Association of Official Seed Analysts in 1931 and 1932 (349, 350), but publication was delayed until 1938. Davis (99) described the haemocytometer method for determining smut spore load in oat seed samples in 1935, and four years later Rice (386) used the same method to measure the fungous spore load in samples of wheat seed. Porter (351) in 1935 described two methods of determining the presence of *Gibberella saubinetii* in seed lots of barley. One method involved planting the seeds on top of moist blotters on large trays, keeping the trays at 20° C. after which the percentage of infected seeds and of blighted seedlings was determined. The scab fungi grew abundantly from infected seeds and were characterized by a grayish-white or pink color. Equally good results were obtained by planting the seeds in copper boxes containing a mixture of autoclaved sand and soil. When many of the seedlings had reached a height of three to five inches, those produced by infected seed developed seedling blight which was characterized by necrosis of the stem from the seed to a half inch above the surface of the soil. Sporulation of the fungus often occurred on the blighted seedlings. In some cases diseased seedlings emerged only slightly or not at all. Germination of 25 samples in the laboratory correlated well with field performance. As a result of this study it was possible to include, in seed laboratory reports of tests, the percentage of seeds that were diseased and to make recommendations for disease control.

A paper published in 1938 by Porter, Hendershott and Davis indicated the relationships of certain seed-borne organisms to germination in laboratory and field. An examination of 129 seed lots of barley, 123 of corn, 75 of flax, 110 of oats and 21 of wheat was made for the presence of seed-borne organisms that affect germination. The principal organisms detected on cereal seeds were *Gibberella saubinetii, Helminthosporium sativum* and *Fusarium* spp. On corn the dry rot fungi *Diplodia, Fusarium* and *Gibberella* were observed and recorded. Laboratory tests with 200 seeds of each lot were made on top of blotters for observation as to parasitic organisms. An equal number was planted in a mixture of autoclaved sand and soil, and counts were made for percentages of normal seedlings and for those with symptoms of seedling blight. An examination of the seeds of barley, oats and wheat was also made for smut spores. Following the laboratory test, all samples were planted in the field with a number of replications, and the percentage germination was determined. Part of the plantings were made in 1933, but most of them in 1934 when the entire middle western section of the United States suffered from a severe drouth. Field germinations were relatively low, yet the correlation between field and laboratory germination was fairly good, in that samples with a low percentage of germinability caused by seedling blight fungi in the laboratory gave a correspondingly low emergence in the field. Records of smutted plants that appeared in the field were made also.

In a second paper published in 1938 Porter (357) gave an outline of the classification of organisms and disease-producing entities carried by seeds and vegetative reproductive organs, described the techniques that were being employed in detecting a large number of saprophytic and parasitic fungi on seeds, and listed the effects of different types of organisms on seed germination or on subsequent plant development. A later paper in 1944 (362) gave a partial list of detectable seed-borne organisms with information as to mode of transmission and symptoms on seeds and seedlings.

Crosier has published a series of papers dealing with the examination of seed samples for seed-borne organisms. He outlined methods (84) that were employed in detecting organisms and reported his results in measuring their pathogenicity. One of his first papers (85) described species of *Fusarium* that occurred on pea seed. He concluded that those species were not aggressive pathogens; they caused no wilt and caused root rot or seed decay only when the mycelium was extremely concentrated and free from other organisms. In 1938 this same author (87) issued a report of his examination of 176 samples of oats and barley collected from farmers' drills and granaries. He found smut spores on the seeds of 174 samples and root-rot and seedling blight organisms on nearly half the samples. Crosier and Weimer isolated fungi from seeds of species of *Poa, Agrostis* and *Festuca,* and tested their pathogenicity. The fungi, in order of their importance as contaminants, were *Curvularia spicifera* (Bainier) Boedijn, *Alternaria tenuis* Nees, *Helminthosporium sativum, H. halodes,* species of *Alternaria, Curvularia, Epicoccum, Fusarium, Helminthosporium* and *Phoma.* Additional fungi listed by Crosier (89) as occurring on seeds are species of *Alternaria, Fusarium* and *Helminthosporium* on *Bromus inermis,* a form of *Diplodia* on cotton, species of *Aspergillus, Cephalothecium, Curvularia, Nigrospora, Penicillium, Rhizoctonia* and *Sclerotium* on peanut, species of *Alternaria, Cladosporium, Curvularia, Myriotleciella* and *Stemphylium* on pumpkin, and a species of *Fusarium* and *Coprinus* on peas and spinach, respectively. Those organisms not reported previously on the respective hosts are listed in the table at the end of this section.

Tullis examined rice kernels for the presence of organisms and identified species of 15 genera which he considered of some importance in relation to discolorations and vitality. The genera that he found were *Alternaria, Aspergillus, Chaetomium, Cladosporium, Curvularia, Epicoccum, Fukelina, Fusarium, Helococeras, Helminthosporium, Monascus, Nigrospora, Phoma, Podoconis* and *Trichoconis.*

Russell and Ledingham used routine laboratory tests of wheat seed to determine the presence of covered smut and of common root-rot organisms. Embryo exposure which permits entrance of fungi was also determined. Simmonds described a laboratory method by which he could detect loose smut fungi in seeds of barley and wheat. He removed the embryos by treatment with solutions of KOH and NaOH, and either dehydrated, cleared in cedar oil and examined them without staining, or embedded, sectioned and stained them before making a microscopic examination. Since the loose smut mycelia are carried internally, only microscopic examination can detect their presence. The alternative is to germinate the seeds under conditions favorable for growth of the pathogen and either wait for heads to appear or examine the young spikes before they emerge from the boot.

Greaney and Machacek conducted an annual survey from 1936 to 1942 to determine the prevalence, severity and distribution of seed-borne diseases of cereals in Manitoba. They made plating tests of the seed and found that smut fungi and species of *Helminthosporium* were the most common and important. *H. sativum* was common on barley, wheat and rye, *H. avenae* Eidam was prevalent on oats and *H. teres* Sacc. was prevalent on barley. Only 3.4% of the wheat samples carried more than a trace of *Tilletia tritici* (Beij.) Wint. and *T. laevis* Kuhn, but 76% of the oat samples carried spores of *Ustilago avenae* (Pers.) Jens. and *U. laevis* K. and S., and 73% of the barley samples carried spores of *U. hordei* (Pers.) K. and S. and *U. nigra* Tapke.

Ray and McLaughlin isolated six species and varieties of *Fusarium* from cotton seed, and Gordon isolated and classified the species and varieties of *Fusarium* that occurred on farm samples of cereal seeds in Canada consisting of nine species and four varieties. All organisms not hitherto reported are included in the table at the end of this section.

Leukel and Martin studied the effect of several seed-borne organisms on the germination of sorghum seed. They reported that species of *Alternaria, Aspergillus, Fusarium, Penicillium* and *Rhizopus* were found on seed in great abundance and, with the exception of *Aspergillus,* when used as inoculum often reduced emergence and caused subsequent seedling blight, especially at 15° and 20° C. Seeds with seed-coat injuries gave ready entrance to fungi, and even saprophytic forms often reduced emergence, presumably by depleting the food supply or by producing toxins. On the other hand, there are instances, as shown by Henry and Campbell, where fungi, normally parasitic in sterile soil, become inactivated in field soils, presumably by the antibiotic action of soil organisms.

Workers in the Iowa State College Seed Laboratory have made isolations of organisms carried by seeds, and the results have been published in reports of the Iowa Agricultural Experiment Station (378). The principal organisms found were *Fusarium graminearum* Schwabe and species of *Alternaria, Curvuluria, Epicoccum, Gibberella, Helminthosporium, Fusarium, Mucor, Nigrospora* and *Rhizopus* on sorghum, one species each of *Curvularia, Epicoccum, Nigrospora* and *Phoma* on oats, one species each of *Curvularia, Epicoccum, Phoma* and *Trichuris* on timothy, one species each of *Actinomucor* and *Trichuris* on red clover, *Colletotrichum glycine* Hori, one species each of *Alternaria, Fusarium, Gibberella* and *Phomopsis* on soybean and a species of *Fusarium* on *Vicia faba* L.

Detection of the fungus responsible for the blind-seed disease of ryegrass on seed samples before and after harvest has been studied by a number of investigators since 1938. Hyde classified the causal fungus as a species of *Pullularia* and described a method for estimating the percentage of infected seed before harvest and its correlation with seed germinability. He collected a few seed heads from scattered parts of a field and then removed a few spikelets from scattered positions on each spike. Microscopic examination of each floret in the selected spikelets revealed the percentage of infection. The symptoms consisted of (*a*) a sticky fluid which contained the conidiophores, (*b*) a solid, waxy secretion, either clear or pale pink, and (*c*) a rusty-red color of the grain which appeared dark under the diaphanoscope. This method was recommended to determine the advisability of harvesting a field. Rose attempted to determine the percentage of infected seed by conidial number counts.

He placed 100 seeds per sample in a test tube with five ml. of water and heated the tube in a water bath for 15 minutes at 80° C. The tube was then shaken for half a minute, and a drop of the solution was taken and a conidial count made with a haemacytometer. His error of estimate for germination was between 18 and 19, and therefore he concluded that the method could not be recommended for harvest-forecast purposes. He considered Hyde's method superior.

Neill and Hyde (319) placed the blind-seed fungus tentatively in the genus *Helotium* and considered that it was allied to *H. herbarum.* Later (320) they concluded that the name of the causal organism should be *Phialea temulenta* (Prill.) Delacr. Noble found large numbers of spores of what she considered *Pullularia pullulans* (DeBary) Berk. on seeds of *Lolium perenne* and *L. multiflorum* when they were placed in water. Infected seeds possessed very low germinability. She stated that the relationship of the observed fungus to the blind-seed disease in Great Britain was yet to be established, and in the following year Wilson, Noble and Gray (492) found that the blind-seed fungus and *Pullularia* sp. are often found on the same seed. Gray named the causal fungus, tentatively, *Phialea mucosa* Gray. Wilson, Noble and Gray (493) described the symptoms of the blind-seed disease, pointed out its distinction from *Pullularia pullulans,* recorded the fact that at least six fungi showing affinities occur on species of *Lolium* and agreed with Neill and Hyde (320) that *P. mucosa* and *P. temulenta* are identical. Calvert and Muskett studied the blind-seed disease in Ireland and came to the same conclusions as Wilson, Noble and Gray, namely, that *P. temulenta,* the causal fungus, is distinct from *Pullularia pullulans* which was found to be non-parasitic. They also isolated the blind-seed fungus from *Festuca ovina* L., *Agrostis canina* L., *A. palustris* Huds., *Poa pratensis, Holcus lanatus* L. and *Cynosurus cristatus* L. Artificial infection of *Festuca elatior* and *Poa trivialis* L. was also obtained. Fischer found the blind-seed disease to be prevalent in Oregon. The apothecial stage of the fungus was found on *Lolium perenne, L. temulentum* L. and *Hordeum Gussoeanum* Parl. The conidial stage was detected on *Agrostis exarata* var. *monolepis* (Torr.) Hitchc., *Aira caryophyllea* L., *Alopecurus geniculatus* L., *Bromus racemosus* L., *Cynosurus echinatus* L., *Deschampsia caespitosa* P. Beauv., *Danthonia californica* Boland, *Festuca elatior* L., *F. myuros* L., *Glyceria* *borealis* (Nash) Batchelder, *H. Gussoeanum, Holcus lanatus* and *Phleum pratense.* Other hosts that have been reported (61) are *Festuca arundinacea* Schreb., *F. rubra* var. *fallax* Hack. and *Lolium multiflorum.*

Meehan (275) made a study of seed samples received by the seed laboratory to determine the organisms carried by seeds of grasses, oats, barley, wheat, clover and soybean. She observed the symptoms of infection on the seeds and seedlings in the germinator, isolated the organisms, identified them and tested their pathogenicity. She made isolations from 25 species of grasses and found 26 species of Dermatiaceae. Of the 271 isolates about 200 belonged to the genus *Helminthosporium* and most of the others to *Curvularia. Helminthosporium victoriae* Meehan and Murphy, a new species, was isolated from seedlings of oats (277). Other organisms which Meehan found are included in the list at the end of this chapter. Later (276) she prepared a host index to the seed-borne species of *Helminthosporium* and *Curvularia* on certain grasses.

The percentage of internal infection in seed corn with various seed-borne fungi was found by Koehler and Dungan to be affected by methods of curing the seed. Percentages were 5.1, 18.3 and 69, respectively, for seed lots dried (*a*) four days at 100° F. and 32% relative humidity, (*b*) four weeks at 70° F. and 65% relative humidity and (*c*) three months at 70° F. and 86% relative humidity. Retarded drying markedly increased the infection percentages by *Fusarium moniliforme* Sheldon, *Nigrospora* spp. and *Penicillium* spp.

Another factor that is closely related to the significance of seed-borne fungi is the age of the seed. Shands found that the longevity of *Gibberella saubinetii* in barley seed was only about 18 months, whereas barley embryos under the same conditions of environment remained viable for longer periods. Dungan and Koehler reported that infection of seed corn by *Gibberella zeae* was almost eliminated by one year in storage and that infection by *Nigrospora oryzae* and *Diplodia zeae* was reduced 50% or more. A marked reduction in the infection of *Cephalosporium acremonium* Corin and *F. moniliforme* occurred within three years. On the other hand, the virus of yellow ring spot of tobacco, according to Valleau (472), survived five and a half years in tobacco seed.

Development of techniques for detecting organisms on seeds in seed testing laboratories may have more far reaching effects in the future than is apparent at present. Seed laboratories receive many kinds of seed each year; hence an excellent opportunity is presented not only to assist in plant disease surveys over a wide area but also to detect the early appearance of new organisms which may become serious if efforts to control them are not launched. To accomplish this possible objective it will be necessary to provide each official seed laboratory with either a trained mycologist or plant pathologist. Seed treatment campaigns have been initiated as a result of seed laboratory tests, thus assisting in disease control. Another possible development is that of determining the presence of seed-borne entities on seed stocks moving in commerce as an aid to plant quarantine regulations. This aspect was discussed by Porter (361) who indicated the types of cooperation between officials of different countries that would be necessary to make such an effort successful. The future holds much promise in both the research and practical aspects of this phase of seed testing.

Lists of Seed-Borne Organisms and Viruses. A valuable compilation of seed-borne organisms including viruses and nematodes was published by Orton in 1931. Since that time a number of investigators have reported additional instances of seed-transmission of disease producing entities. Groves and Skolko, Neergaard (317) and Meehan (275) have described the seed-borne fungi isolated by them, and their publications are of value to others who wish to make comparisons for purposes of identification.

The following list of seed-borne organisms and viruses includes, with a few exceptions, those which have been reported since Orton's publication, insofar as the writer has been able to compile them. The exceptions* are those for which some additional evidence of seed-transmission has been supplied or which have been reported from parts of the world not hitherto included in Orton's list. There are undoubtedly some ommissions which have escaped attention, but it is hoped that the list will be of assistance to phytopathologists and seed analysts.

USE OF SEED FUNGICIDES IN SEED TESTING

Treatment of seeds with fungicides as a part of the practice of seed testing is a recent development which took place almost simultaneously with that of detection of seed-borne organisms. It

SEED-BORNE ORGANISMS AND VIRUSES, AS REPORTED SINCE 1931

Organism or Virus	Host	By Whom Isolated
1. Bacteria		
**Phytomonas aptata* (Brown and Jamieson) Bergey *et al*	Sugar Beet	Ark and Leach
P. incanae Kendrick and Baker	*Methiola incana*	Kendrick and Baker
**P. malvacearum* (E. F. Smith) Bergey *et al*	Cotton	Hare and King
2. Viruses		
Ring spot	Tobacco	Valleau
Yellow ring spot	Tobacco	Valleau
Streak and mosaic	Tomato	Berkeley and Madden
Yellow dwarf	Onion	Henderson
3. Fungi		
Acremoniella atra (Corda) Sacc.	Agricultural seed	Groves and Skolko (160)
A. verrucosa Togn.	Agricultural seed	Groves and Skolko (160)
Actinomucor sp.	Red clover	Rice and Meehan (378)
Alternaria brassicae (Berk.) Sacc.	*Iberis umbellata*	Neergaard (317)
Alternaria brassicae	*Lepidium sativum*	Neergaard (317)
A. brassicae (Berk.) var. *dauci* (Kühn) Bolle.	Carrot	Neergaard (317)
A. cheiranthi (Fr.) Bolle.	Wallflower	Neergaard (317)
A. circinans (Berk. and Curt.) Bolle.	*Iberis umbellata* and radish	Neergaard (317)
A. dianthi Stev. and Hall	Carnation	Neergaard (317)
A. linicola Groves and Skolko	Flax	Groves and Skolko (158)
A. oleracea Milbr.	Cruciferae and *Phaseolus coccineus*	Groves and Skolko (158)
A. porri (Ell.) Neerg.	*Allium* spp.	Neergaard (317)
A. radicina Meier, Dreschler and Eddy	Squash, carrot, parsley	Groves and Skolko (158)
A. raphani Groves and Skolko	Radish	Groves and Skolko (158)
A. rugosa McAlpin	*Schizanthus hybridus*	Neergaard (317)
A. tenuis Nees.	*Poa, Agrostis, Bromus, Festuca*	Crosier and Weimer
A. tenuis	Grasses and agricultural seed	Groves and Skolko (158)
A. tenuissima (Fr.) Wiltsh.	*Godetia hybrida*	Neergaard (317)
A. sp.	Pumpkin	Crosier (89)
A. sp.	Soybean	Tervet

Organism or Virus	Host	By Whom Isolated
A. spp.	Sorghum, soybean	Porter, Rice and Meehan (378)
A. spp.	Rice, sorghum	Tullis, Leukel and Martin
A. sp.	*Gypsophila elegans*	Neergaard (317)
A. sp.	*Zinnia elegans*	Neergaard (317)
Ascochyta caulicola Laub.	Sweet clover	Jones (207)
A. imperfecta Pk.	Alfalfa	Cormack
A. lactucae Restr.	Lettuce	Neergaard (317)
A. pseudo pinodella Bond and Vass.	Pea	Bondortzeva-Monteverde and Vassilievsky
Aspergillus flavus Link	Soybean	Tervet
A. niger Van Tieghem	Soybean	Tervet
A. ochraceous Wilhelm	Soybean	Tervet
A. sp.	Peanut	Crosier (89)
A. spp.	Rice, sorghum	Tullis, Leukel and Martin
**Botrytis anthophila* A. Bond	Red clover	Silow
B. bryssoidea Walk	Allium	Neergaard (317)
B. cinerea Pers.	Cabbage, lettuce, pea, red clover	Doyer (117)
B. cinerea Pers.	*Scorzonera hispanica*, spinach	Doyer (117)
B. cinerea Pers.	*Clarkia elegans*, *Godetia hybrida*	Neergaard (317)
B. cinerea	*Primula*, rhubarb, zinnia	Neergaard (317)
B. trifolii Van Beyma	Red clover	Doyer (116)
Brachycladium sp.	*Sporobolus* sp.	Meehan (275)
Brachysporium obovatum	*Lolium perenne*	Meehan (275)
Cephalosporium sp.	Red clover	Meehan (275)
Cephalothecium sp.	Peanut	Crosier (89)
Cercospora carotae (Pass.) Solheim	*Daucus* sp.	Thomas
C. zebrina Pass.	Sweet clover	Jones (208)
Chaetomium sp.	Rice	Tullis
Chlamydomyces palmarum (Cooke) Mason	Parsnip, pea	Groves and Skolko (160)
Cladosporium herbarum (Pers.) Lk.	Rice, *Thesium*	Tullis, Chabrolin
C. pisicolum Snyder	Pea	Snyder
Colletotrichum circinans (Berk.) Vogl.	Onion	Neergaard (317)

Organism or Virus	Host	By Whom Isolated
C. glycine Hori	Sovbean	Porter, Rice and Meehan (378)
C. lineola Cda.	Sorghum	Porter (357)
C. spinaciae E & H	Spinach	Doyer (117)
Coprinus lagopus Fr.	Spinach	Crosier (89)
Corticium solani (Kuhn) Burt	Pea, spinach	Neergaard (317), Crosier (89)
Curvularia geniculata (Tracy & Earle) Boedijn	Cabbage, flax, pea	Groves and Skolko (159)
C. geniculata	*Agropyron, Avena, Axonopus, Bouteloua,* sorghum and other grasses	Meehan (275)
C. inaequalis (Shear) Boedijn	Flax, grasses, pea, turnip, spinach	Groves and Skolko (159)
C. inaequalis (Shear) Boedijn	Peanut	Prince
C. inaequalis	*Avena, Axonopus, Bouteloua, Paspalum,* timothy and other grasses	Meehan (275)
C. intermedia Boedijn	Timothy	Meehan (275)
C. lunata (Wakker) Boedijn	Pumpkin, rice	Crosier, Tullis
C. lunata	Onion, pepper	Groves and Skolko (159)
C. lunata	*Agropyron, Agrostis, Bouteloua, Chloris, Dactylis,* rice and timothy	Meehan (275)
C. maculans (Bancroft) Boedijn	Rice	Tullis
C. maculans	Timothy	Meehan (275)
C. pallescens Boedijn	Cauliflower, turnip	Groves and Skolko (159)
C. pallescens	*Axonopus*	Meehan (275)
C. spicifera (Bainier) Boedijn	*Poa, Agrostis, Festuca*	Crosier and Weimer
C. spicifera	*Avena, Axonopus, Bouteloua, Buchloe, Chloris, Dactylis, Hordeum, Paspalum, Triticum*	Meehan (275)
C. trifolii (Kauffman) Boedijn	Timothy	Meehan (275)
C. sp.	Peanut	Crosier (89)
C. sp.	Sorghum, timothy	Porter, Rice and Meehan (378)

Organism or Virus	Host	By Whom Isolated
Dendryphium penicillatum (Corda) Fr.	Poppy	Neergaard (317)
D. spp.	Poppy, meadow fescue	Doyer, Meehan (275)
Didymella lycopersici Kleb.	Tomato	Alcock
Diplodia natalensis Evans	Peanut	Prince
Epicoccum neglectum Desm.	Rice	Tullis
E. purpurascens Ehrbg.	*Poa, Agrostis, Festuca*	Crosier and Weimer
E. sp.	Sorghum, timothy	Porter, Rice, Meehan (378)
Erysiphe cichoracearum DC.	Zinnia	Baker and Locke
E. sp.	Parsley	Doyer (117)
Fusariella sp.	Peanut	Prince
Fusarium avenaceum (Fr.) Sacc	Carrot	Neergaard (317)
F. chlamydosporum Wr. and Rg.	Cotton	Ray and McLaughlin
F. chlamydosporum	Peanut	Prince
F. concolor Rg.	Barley	Gordon
F. culmorum (W. Sm.) Sacc.	*Poa, Agrostis, Festuca*	Crosier and Weimer
F. equiseti (Cda.) Sacc.	Cotton	Ray and McLaughlin
F. graminearum Schwabe	Cereals	Gordon
F. graminearum	Sorghum	Porter, Rice, Meehan (378)
F. lateritium Nees.	Peanut	Prince
F. lateritium var. *uncinatum* Wr.	Peanut	Prince
F. lycopersici Brushi	Tomato	Kendrick (222)
F. moniliforme Sheld	*Poa, Agrostis, Festuca*	Crosier and Weimer
F. moniliforme	Peanut	Prince
F. niveum E. F. Sm.	Watermelon	Taubenhaus
F. orthoceras App. and Wr. var. *pisi* Linford	Peas	Snyder
F. oxysporum Schlecht.	Cereals	Gordon
F. oxysporum	Peanut	Prince
F. oxysporum	Peas	Crosier (89)
F. poae (Pk.) Wr.	Cereals	Gordon
F. reticulatum Mont.	Peanut	Prince
F. sambucinum Fuck. var. *coeruleum* Wr.	Cereals	Gordon
F. sambucinum form 6	Durum wheat	Gordon
F. scirpi Lamb. and Fautr.	Cotton	Ray and McLaughlin

Organism or Virus	Host	By Whom Isolated
F. scirpi Lamb. and Fautr. var. *acuminatum* (Ell. and Ev.) Wr.	Cotton	Ray and McLaughlin
F. scirpi Lamb. and Fautr. var. *acuminatum* (Ell. and Ev.) Wr.	Cereals	Gordon
F. scirpi Lamb. and Fautr. var. *caudatum* Wr.	Poppy	Christoff
F. scirpi Lamb. and Fautr. var. *compactum* Wr.	Cereals	Gordon
F. semitectum Berk. and Rav.	Cereals	Gordon
F. semitectum	Cotton	Ray and McLaughlin
F. solani (Mart.) App. and Wr.	Cotton	Ray and McLaughlin
F. solani f. *eumartii* (Carp.) Wr.	Peanut	Prince
F. sporotrichoides Sherb.	Cereals	Gordon
F. tracheiphilum E. F. Smith	Cowpea	Kendrick (223)
F. vasinfectum Atk.	Okra	Taubenhaus
F. sp.	*Bromus inermis*	Crosier (89)
F. sp.	Cantaloupe	Leach
F. sp.	Lettuce	Doyer (117)
F. sp.	Red clover	Doyer (117)
F. sp. (wilt)	Beans	Kendrick
F. sp.	Sorghum	Porter, Rice and Meehan (378)
F. sp.	Sorghum	Leukel and Martin
F. sp.	Soybean	Porter, Rice and Meehan (378)
F. sp.	Soybean	Tervet
F. sp.	*Vicia Faba*	Porter, Rice and Meehan (378)
F. spp.	Peanut	Prince, Wilson (489)
F. spp.	Rice, peanut	Tullis, Wilson (489)
F. spp.	Spinach	Doyer (117)
Fukelina sp.	Rice	Tullis
Gibberella sp.	Sorghum	Porter, Rice and Meehan (378)
G. sp.	Soybean	Porter, Rice and Meehan (378)
Gliocladium catenulatum Gilman and Abbott	Peanut	Prince
Helicoceras oryzae Linder and Tullis	Rice	Tullis
Helminthosporium catenarium Drechsl.	*Buchloe, Festuca,* timothy	Meehan
H. dictyoides Drechsl.	*Buchloe, Festuca*	Meehan

Organism or Virus	Host	By Whom Isolated
H. halodes Drechsler	*Poa, Agrostis, Festuca*	Crosier and Weimer
H. oryzae Van Breda de Haan	*Festuca*, timothy	Meehan (275)
H. rostratum Drechsl.	*Axonopus, Bouteloua, Chloris Cynodon, Paspalum*, timothy *Poa*	Meehan (275)
H. sativum P. K. and B.	Bromegrass	Crosier (89)
H. sativum	*Agropyron, Buchloe*, timothy, sorghum, *Dactylis*	Meehan (275)
H. triceptatum Drechsl.	*Agrostis*, timothy	Meehan (275)
H. turcicum Pass.	Sudan grass	Chilton (66)
H. victoriae Meehan and Murphy	*Agropyron, Avena, Chloris, Dactylis, Festuca, Paspalum*, timothy, *Setaria*, sorghum	Meehan (275)
H. sp.	Sorghum	Porter, Rice and Meehan (378)
Leptophaeria pratensis Sacc. and Briard	Sweet clover	Jones (208)
Macrophomina phaseoli (Maubl.) Ashby	Bean	Andrus
Macrosporium sp.	Red clover	Doyer (117)
Miriotleciella sp.	Pumpkin	Crosier
Monascus purpurescens Went, Hedw.	Rice	Tullis
Mucor sp.	Sorghum	Porter, Rice and Meehan (378)
M. sp.	Peanut	Prince
Mycosphaerella lethalis Stone	Red clover	Jones (208)
Nigrospora oryzae (B. and Br.) Petch	Rice	Tullis
N. sp.	Peanut	Crosier (89)
N. sp.	Sorghum, oats	Porter, Rice and Meehan (378)
Penicillium sp.	Sorghum	Leukel and Martin
P. sp.	Soybean	Tervet
P. sp.	Peanut	Prince
Peronospora arborescens (Ber.) de Bary	*Meconopsis*	Alcock
P. effusa (Grev.) Rbh.	Spinach	Cook
P. parasitica (Pers.) Tul.	Cabbage	Alcock
**P. schachtii* Fckl.	Beet	Leach (242)
P. viciae Berk.	Pea	Alcock

Phialea temulenta (Prill.) Delacr.	*Lolium* and other grasses	Gray, Neill and Hyde, Calvert and Muskett, Fischer
Phoma glomerata (Cda.) Wright and Hochapfel	Grasses	Crosier (89)
**P. insidiosa* Tassi	Sorghum	Porter (357)
P. nemophilae Neerg.	*Nemophila* spp.	Neergaard (317)
P. rhoedis Brun.	Poppy	Neergaard (317)
**P. restrupii* Sacc.	Carrot	Jorgensen
P. siliguae Sacc.	Wallflower	Neergaard (317)
P. sp.	Spinach	Doyer (117)
P. sp.	*Valerianella olitaria*	Doyer (117)
P. spp.	Rice	Tullis
Phyllosticta acetosa Sacc.	*Rumex*	Neergaard (317)
P. primulicola Desm.	*Primula*	Neergaard (317)
Phytophthora infestans (Mont.) De By.	Tomato	Boyd
Pleospora calvescens (Fr.) Tul.	Poppy	Christoff
P. herbarum (Pers.) Rbh.	Bean, onion	Doyer, Alcock
P. herbarum	Many agricultural seeds	Groves and Skolko (157)
Podoconis sp.	Rice	Tullis
Puccinia porri (Sow.) Winter	*Allium*	Alcock
Pullularia pullulans (De Bary) Berk.	*Lolium*	Noble
Ramularia sp.	*Parthenium argentatum*	White
Rhizopus artocarpi Rac.	Peanut	Prince
R. solani Kühn	Subterranean clover	Chilton (67)
R. solani	Peanut	Prince
R. sp.	Peanut	Crosier (89)
R. spp.	Peanut	Wilson
Rhizopus nigricans Ehbg.	Peanut	Prince
R. sp.	Sorghum	Leukel and Martin
R. sp.	Peanut	Prince
Sclerochaeta sp.	Peanut	Prince
***Sclerospora graminicola* (Sacc.) Schroet.	Millet	Porter (357)
Sclerotinia sclerotiorum (Lib.) Schroet.	Bean, *Scorzonera hispanica*	Doyer (17)
S. sclerotiorum	Carrot, dahlia	Alcock

S. sclerotiorum	Pea	Crosier (89)
S. trifoliorum Eriks.	Red clover	Doyer (117)
Sclerotium bataticola Taub.	Peanut	Prince, Wilson (489)
S. sclerotiorum (Lib.) Mass.	Sunflower	Moore
Septoria petroselini Desm.	Parsley	Doyer (117)
Sphacelotheca reiliana (Kuehn) Clint. (*Sorosporium reilianum* (Kuehn) McAlpine)	Corn	Bressman and Barss.
Sphaeronema sp.	Cabbage	Doyer (117)
Stagonospora meliloti (Lasch.) Petr.	Sweet clover	Jones (209)
Stagonosporopsis hortensis (Sacc. and Mulbr.) Petr.	Bean	Doyer (117)
Stemphylium consortiale (Thüm) n. combr.	Many agricultural seeds	Groves and Skolko (157)
S. radicinum (M. D. and E.) Neerg.	Carrot	Doyer (117)
S. radicinum	Celery	Neergaard (317)
S. radicinum var. *petroselini* Neerg.	Parsley	Neergaard (317)
S. sarcinaeforme (Cav.) Wilts.	Red clover	Chilton
S. sp.	Pumpkin	Crosier
Trichocladium aspermum (Cda.) Harz.	Carrot, lettuce, pea, pumpkin, *Vicia Faba*	Groves and Skolko (160)
Trichoconis caudata (Ap. and Str.) Clem.	Rice	Tullis
Trichoderma viride Fr.	Peanut	Prince
Trichuris spp.	Red clover, timothy	Porter, Rice and Meehan (378)
Typhula trifolii Rostr.	Red clover	Doyer (117)
Uromyces betae (Pers.) Tul.	Beet	Alcock
Ustilago tragopogi-pratensis (Pers.) Rouss.	Salsify	Alcock
Verticillium sp.	Eggplant	Kadow

is natural that these two aspects of seed technology should have been emphasized together, but the rapid development of dust fungicides no doubt accelerated their use in seed testing. It is not the purpose of this chapter to review the extensive literature dealing with the widespread use of seed disinfectants as one method of disease control, but rather to review only those papers which bear, more or less directly, on seed testing in both its research and practical aspects. Developments in this field have included (*a*) laboratory and field germination of treated and untreated seed known to be infected with specific organisms and (*b*) treatment with disinfectants to observe their effect on parasitic and saprophytic organisms carried by seeds. These two phases are treated together.

A beginning in the use of seed disinfectants in connection with seed testing was made in 1928 (349) when samples of farmer's seed corn were ear-tested and classified as nearly disease-free or infected with dry rot fungi. A third class was prepared by visually selecting, from early picked seed, ears that appeared relatively free from disease. All three classes were then divided into two subclasses, one treated, the other untreated, and all were planted in the field where counts for germination were made and yields were recorded. The results showed that visually selected treated seed yielded as well as nearly disease-free seed either treated or untreated. These results were presented at the Annual Meeting of the Association of Official Seed Analysts in 1930, but publication was delayed until 1938. This work with farmers' seed corn marked the beginning of an intensive educational campaign in Iowa for early selection, careful drying, dry storage, ear selection followed by a representative germination test and treatment of seed corn with mercury dust fungicides as a substitute for wholesale ear testing.

Further studies were initiated in 1932 and 1933 with seeds of barley, corn, flax, oats and wheat, from which several publications followed. The first one dealt with laboratory and field germination of barley seed (351) infected with *Gibberella saubinetii* and *Helminthosporium sativum*. Untreated and treated seeds of lots with varying degrees of infection were planted on blotters, in a mixture of sand and soil and in the field. The percentages of infected seeds and seedlings, of normal sprouts and of blighted seedlings were recorded from the laboratory tests. Percentage germination in the field was determined and recorded. One interesting feature of the investigation was the use of 5% ethyl mercury phosphate (New improved Ceresan) diluted with talc to make a 1% dust. Treatment with the ethyl mercury phosphate was thus facilitated by applying a slight excess of the talc-phosphate mixture and removing the excess by shaking the treated seed in a tea strainer. The higher percentage of germination from treated than untreated seed obtained in the laboratory was duplicated in the field.

The results of subsequent tests published in 1938 (366) involved seeds of barley, corn, flax, oats and wheat, a total of 458 samples of farmers' seed infected in varying degrees with seed-borne organisms. In most cases there was a good correlation between the germination of samples in the field and laboratory, and the higher percentage germination of treated than untreated seed was relatively maintained under both sets of conditions. Three types of diluents for 5% ethyl mercury phosphate were used, namely, talc, gypsum and button dust. Gypsum alone exhibited some germicidal value on cereal seeds infected with *G. saubinettii.* Yield results from all treated and untreated seed lots were also obtained. The data resulting from this study illustrated the applicability of such pathological techniques as detection of seed-borne organisms and the effect of disinfectants on seed germination to seed laboratory practice, in that the laboratory tests were reasonably indicative of field response.

In a paper (353) presented at the Annual Meeting of the Association of Official Seed Analysts in 1936 the use of seed disinfectants in seed laboratory practice was described as a practical measure for teaching an important method of disease control to recipients of seed laboratory reports. This was accomplished by stating the germination of treated and untreated seed on the same report, as well as the percentage of diseased seeds or seedlings for such crops as barley, corn, flax, oats, peas and wheat. The dusts were applied in the laboratory by placing the seeds with the proper amount of dust in a bottle which was rotated on two rollers, one of which was driven by an electric motor. Mercury poisoning, which results from an excess of volatile mercury compounds applied to seeds, was also illustrated. Sass found that over-doses of ethyl mercury phosphate applied to certain seeds caused giant nuclei which resulted in some lateral growth but no elongation of the plumule.

A possible classification of seed fungicides, their use in seed research and seed laboratory practice for the control of parasitic and saprophytic organisms and their effect on seed germination in laboratory and field was published in 1938 (357). The seeds that were studied included lima beans, peas, sorghum and soybean, in addition to those previously studied.

Arasan, Ceresan and Spergon were applied to five lots of pea seed, and Arasan, Semesan J. and Spergon were applied to 22 lots of sweet corn and six lots of popcorn planted on cellucotton or blotters (364). All treatments reduced the percentage of decayed peas, gave almost complete control of a species of *Fusarium* and largely eliminated infection by saprophytic fungi. The treatments applied to sweet corn gave good control of *Fusarium* spp. and fairly good control of saprophytes, but Arasan was superior to both Semesan Jr. and Spergon. The percentages of seed infected with saprophytic forms were 67, 13.1, 21.7 and 22.7 for untreated and Arasan, Semesan Jr. and Spergon treated, respectively. On popcorn seed all three treatments gave satisfactory control of parasitic and saprophytic fungi.

Crosier and his associates in New York have made a series of contributions toward the use of disinfectants in a seed laboratory for the control of parasitic and saprophytic fungi. One of the earliest papers described the abnormal germination of wheat seed caused by organic mercurials (86). Crosier and Nelson used Barbak C., Ceresan, $HgCl_2$ and New Ceresan on lima bean seed with resultant control of contaminating micro-organisms during the germinating period. Crosier and Patrick (92) investigated the effect of hot water and chemical dusts on seeds of cabbage, radish and broccoli infected with *Alternaria* spp. They found that the beneficial action of seed treatment is dependent on the amount of seed contamination by both *Alternaria* and *Rhizopus*. The mercurial disinfectants effected partial control, but Cu_2O and ZnO were less effective. Removal of excess mercurial dusts from the seeds was considered necessary. Crosier (88) treated seed of cucumber, muskmelon and squash, prior to germination, with Spergon, United States Rubber Co. materials No. 601 and No. 604, and with the ethyl group of mercurials. Spergon was excellent for the control of damping off, but was considered a poorer fungicide than the ethyl mercury compounds. A referee test among 11 official seed testing stations

(90) was conducted, using pea seed and Stowell's Evergreen sweet corn seed infected with *Diplodia zeae* and other mold fungi, treated with various chemicals in comparison with untreated seed. Arasan excelled in the control of *Diplodia zeae* and Fusaria; and No. 1452 C in the control of *Rhizopus nigricans,* in seed germination and in green weights of seedlings of sweet corn. Arasan was superior to Semesan Jr., Ceresan, Spergon and No. 604 in the increase over untreated seed of green weight of seedlings. Spergon proved superior in the control of *R. nigricans* and in percentage of germinated seed, and No. 604 gave better control of bacteria and other mold fungi. Crosier and Patrick (93) reported that Arasan alone or mixed with flour applied to ten lots of sweet corn seed retarded the growth of *D. zeae,* Fusaria and *R. nigricans* and that the seedlings from Arasan-treated seed weighed more than those from untreated seed.

Doyer in Holland has reported the use of seed disinfectants in connection with seed testing. She treated flax seed (115) with Tillantin R. which gave satisfactory control of *Botrytis cinerea* f. *lini* and *Colletotrichum linicolum.* The compound U. T. 685 dusted on beet seed controlled *Phoma betae.* Clover seed (116) infected with species of *Botrytis, Macrosporium, Sclerotinia* and *Typhula* was treated with Germisan and Ceresan which gave good control. Various seed-borne diseases of vegetables were described in 1941 (118), and methods of seed disinfection were given. In one instance untreated lettuce seed with a germination of only 29% gave 76% germination after treatment with Ceresan.

One of the most practical results of treating seeds prior to a germination test is that it can promote more uniformity in the evaluation of seed lots. When samples are overrun with saprophytic organisms or severely infected with parasitic species it becomes difficult to evaluate a germination test. Yet this difficulty can usually be overcome by proper seed treatment. A referee test (379–1938: 52) conducted in 1938 with treated and untreated seed of barley and wheat demonstrated in a remarkable way the uniformity of interpretation of normal seedlings by five different stations when the seed was treated with ethyl mercury phosphate. Evaluation of seedlings produced by the untreated seed was highly variable. On the other hand, no laboratory should report a test of treated seed without a corresponding test of untreated seed unless the sample was treated before the laboratory received it. The reason for this precaution is obvious.

Finally the ultimate application of seed disinfection to seed testing is dependent on the extent to which commercial lots of seed are treated with disinfectants as a part of seed processing prior to sale. There is a growing need and demand for treated seed of many field and vegetable crops. As more non-poisonous compounds become available and mailing restrictions are modified, more and more seed will be sold as treated seed. Seed laboratories that receive untreated samples which are to be marked as treated seed will be justified in treating a portion of the sample prior to making a viability test. Familiarity with different seed treatment compounds will thus become a further necessary requirement of seed technologists.

DEVELOPMENT OF LABORATORY METHODS FOR MEASURING SEED GERMINABILITY UNDER ADVERSE CONDITIONS

The discussion in Chapter 5 of this paper pointed out that the criterion for measuring seed germinability has been the percentage of normal seedlings which are produced under optimum conditions when such are known for each kind of seed. It has been recognized that field germination, except under a very favorable environment, cannot be expected to equal that in the laboratory. Three developments within the past few years have served to indicate the desirability of knowing in advance of planting how certain kinds of seed may be expected to germinate in an environment considerably below the optimum. In the first place there has been a pronounced interest among phytopathologists in seedling blight and damping-off caused by soil-borne fungi. A study of these conditions has revealed valuable information relative to the interrelationship of the temperature, moisture and fungous flora of the soil in their effect on germinating seeds and on seedlings. In the second place the marked production of new seed protectants has made it necessary to develop laboratory methods for their evaluation. In the third place plant breeders have produced a tremendous number of new crop varieties by hybridization, especially of field and sweet corn. This program has not only created innumerable genetic combinations in crop plants but has also resulted in large scale commercial production of expensive seed stocks that have required artificial drying and more mechanical processing methods than were previously employed. Field experience, especially earlier planting than in previous years, has revealed striking differences in germinability of seed stocks which were not detectable under favorable laboratory conditions.

It is neither the purpose nor function of this present paper to review the voluminous literature dealing with soil fungi, the innumerable field tests with seed fungicides or the production of new varieties and hybrids but rather to limit the review to a few publications which have a pertinent relationship to this new phase of seed laboratory practice. This review covers not only the germination of untreated seed under adverse conditions in laboratory and field but also the comparative value of seed protectants under the same conditions.

Horsfall has made valuable contributions to our knowledge of the factors that determine the extent of pre-emergence and post-emergence damping-off of seedlings as well as the relative benefits of several seed protectants. In 1932 (190) he published his results with red oxide of copper (Cu_2O) as a seed treatment to combat damping-off of several kinds of vegetable crop seedlings. He made plantings in greenhouse flats, using soil infested with *Pythium ultimum* Trow. Cu_2O was found to be especially valuable for smooth seeds such as pepper and egg-plant because of its adhesive qualities, and on tomato seeds it adhered two and a half times as well as monohydrated copper sulfate. On the basis of copper content the adhesiveness was seven times as great. His paper published in 1938 (191) summarized nine years of work on damping-off and its control by seed protection and by soil treatments. One of the important conclusions was that high soil moisture seems to enhance pre-emergence damping-off more than low soil temperatures. He also investigated inoculum potential or disease-producing power of the soil in relation to seed protection. When the inoculum potential exceeded 80% of the maximum the protective power of Cu_2O and $CuSO_4$ fell off rapidly. He calculated that 0.5 mg. of metallic copper (as cuprous oxide) per sq. cm. of seed surface is required to give minimum coverage dosage on seed, without regard to its kind.

The comparative germination of treated and untreated pea seed in auto-claved and non-auto-claved soil was studied by Davis (101) who found a marked difference in seedling emergence from untreated seed planted in the two kinds of soil. In non-auto-claved soil the percentage emergence was severely reduced by the attack of soil fungi, and seed treatment provided an effective control against the seed-rotting organisms. Ogilvie *et al* treated peas with Cu_2O and found that under certain environmental conditions in the greenhouse and field it increased the emergence of seedlings, although it sometimes retarded the rate of emergence. In dry soils Cu_2O caused injury to seed of some varieties.

Reddy and Rice reported that seed corn which gave high germination under favorable conditions in the laboratory often germinated much less when planted in moist unsterilized soils kept at a low temperature. Seed treatment proved beneficial under adverse conditions. A tensiometer designed by the junior author was of value in regulating the amount of water in the soil for any specific test. Tatum and Zuber used the method developed by Rice, as employed in the Iowa State College seed laboratory, to test the germination of corn that had received different processing treatments. Their results showed that under adverse conditions (low temperature in non-sterile soil) marked injury to germination of seed corn occurred. Machacek and Wallace considered that non-sterile soil served as a good medium in which to test the response of cereal seeds prior to field planting. The results obtained were considered more indicative of probable field performance. Leukel and Martin used soil artificially infested with several species of soil- and seed-borne fungi to test the germination of sorghum seed.

Rice (387) made an extensive investigation of the germination of many field crop seeds in *Pythium*-infested soil mixed with different amounts of sand. He varied the temperature and the amount of water that was maintained in the soil and planted untreated seed and seed treated with many different commercial fungicides. Samples of corn of different ages and freshly-harvested corn dried by different methods as well as other crop seds were used in his investigations. Planting dates in the field varied from very early to the normal planting time. He used a tensiometer designed by himself and produced in the College Instrument Shop to measure the water tension in soil which served as an indicator of moisture content. His results showed that (*a*) the moisture content of *Pythium*-infested soil mixed with sand had a marked effect on the percentage of germination and normal seedlings of different kinds of field crops, (*b*) the length of time of exposure of seeds to low temperatures was an important factor in determining the amount of seed decay and damping-off, (*c*) exposure of corn, soybean, sorghum and flax seed at 10° C. in *Pythium*-infested soil for five to ten days followed by a more favorable temperature gave a fair indication of germination under adverse conditions, and (*d*) the relative protective power of seed fungicides could be measured by treating seeds and planting them in moist soil kept at 5° to 10° C. for a part of the germination time. In an experiment station report (362) in 1943 the mean percentage germination of 177 lots of hybrid corn tested in sterile sand at a daily temperature range of 68° to 86° F. was reported as 92. The same samples germinated in *Pythium*-infested soil and sand at 10° C. for seven days followed by a temperature of 26° to 28° averaged 67.5% germination.

Leukel tested 19 materials on a number of varieties of sorghum seed to determine their effect on seedling emergence in sterilized and non-sterilized soil and in the field. He concluded, among other things, that soil temperature and moisture have an important influence on the emergence of seedlings from treated and untreated seed. In general, when seeds were planted in soils infested with virulent fungi, the absolute increase in emergence from treated seed was greatest at 20° C. and least at 15° C., whereas the relative increase was greatest at 15° C. and least at 25° C.

Leach (244) concluded that growth rates of host and pathogen are primary factors that determine the severity of pre-emergence damping-off. Leach and Smth suggested that emergence rates could be measured by calculating a mean emergence period. In their work with seed protectants for peas they stated that comparisons of emergence rates should be limited to trials in sterilized soil or between treatments having equal protective effects. They showed that in pasteurized soil none of the seed treatments tested on disease free pea produced more rapid germination or larger seedlings than the non-treated controls; in fact, yellow cuprous oxide not only delayed emergence and water absorption by the cotyledons but produced necrotic areas on the surface of the cotyledons. In soils heavily infested with *Pythium ultimum*, Semesan, yellow copper oxide and dichloro-naphtha-quinone were among the most effective materials used for the control of pre-emergence damping off. The benefits of seed treatment were considered as entirely due to disease prevention.

Taylor and Rupert used *Pythium*-infested soil in which to plant vegetable seeds in greenhouse flats. They used treated and untreated seed and observed the healthy and damped-off seedlings. Arasan and Fermate gave better control of pre-emergence and post-emergence damping-off than Cuprocide, Semesan and Spergon.

A recent publication by Porter (364) contains a considerable body of data bearing on the germination of vegetable crop seeds under adverse conditions in both laboratory and field. Two methods of testing seed samples were employed in the laboratory, one of which involved optimum, the other adverse conditions. The latter method was basically the same as described and used by Rice (387) in that the laboratory medium consisted of two parts sand and one part *Pythium*-infested soil, and the temperature used was 10° C. for different periods of time, depending on the kind of seed. The tests under optimum conditions were made to observe the seeds and seedlings for symptoms of parasitic and saprophytic organisms as well as to determine maximum viability. The tests under adverse conditions employed both treated and untreated seed, and plantings were made in the field to compare the results with those in the laboratory. In many cases field conditions were adverse for germination, but not in every instance. This investigation involved 50 samples of peas, 41 of sweet corn, six of popcorn, five of soybean, 14 of spinach, 11 of onion, six of beet, five of carrot, one of tomato and nine of bush lima and green pod beans. The data obtained by this study revealed that by planting untreated and treated vegetable seeds under standardized adverse conditions it was possible to,

(*a*) index samples and varieties as to their resistance or susceptibility to seed decay and damping-off;

(*b*) measure the relative resistance to adverse germinative conditions of old and new crop seed of the same variety, which gives an indication of physiological decline in vitality resulting from age of seed;

(*c*) obtain a fair index of expected germination of seed lots planted under adverse field conditions;

(*d*) index the relative effectiveness of seed protectants for the control of seed decay and damping off.

This particular study and a previous article (363) also revealed that the cold soil treatment may induce a pathological type of baldhead in soybean and lima bean seedlings, a condition which resembles that attributed to mechanical injury of seed during threshing (46, 168). Arasan provided an effective control of pathological baldhead in cold soils.

APPLICATION OF STATISTICAL METHODS TO SEED TESTING

The application of statistical methods to the design of experiments and to the analysis and interpretation of biological data has received unusual emphasis the past 15 to 20 years. Fortunately the science of seed testing has been included in this emphasis, particularly within the past decade. Snedecor, by his text book, has provided a useful guide, and Leggatt (251, 252) has made major contributions to this important aspect of seed testing. Others have demonstrated some of the practical applications of statistical science to seed technology.

Leggatt's review (252) is of particular significance because it deals specifically with problems in seed technology from both the theoretical and applied standpoints. A comprehensive review of his paper is unnecessary. His major contributions have dealt with:

(*a*) distribution of small, rough and smooth seeds in bulk lots with different sizes and seed coat characteristics from which he has been able to estimate the size of sample necessary to determine, on a practical basis, the distribution of weed seeds in crop seed or the percentages of different kinds of seeds in mixtures;

(*b*) evaluation of germination tests made by different analysts or by one analyst who compared two or more methods of testing one sample or one method with different samples; he prepared a set of tables to be used in comparing differences between replicate tests when a different number of seeds were used in each replicate;

(*c*) evaluation of different methods of sampling seeds in bags with the objective of obtaining representative samples for analysis;

(*d*) preparation of tolerance tables to be used in making comparisons between replicate purity analyses from a given lot of seed or in estimating the reliability of separate reports on the rate of occurrence of weed seeds in a given lot.

Woodbridge studied the distribution of the seeds of *Cirsium arvense, Rumex crispus* and *Barbarea vulgaris* in timothy and concluded that the latter two became consistently distributed according to the Poisson series, but seeds of *C. arvense* did not. She concluded that 50 grams of timothy seed was needed to determine the content of the three weed species within allowable limits if the number exceeded 30 per 50 grams.

Porter and his associates (354, 356, 358, 362, 366) have applied the chi-square test for homogeneity, the analysis of variance and the coefficient of variability extensively in their analyses of laboratory and field data. These techniques have been of particular value in (*a*) determining the degree of natural variation in purity of small sub-samples drawn from a well mixed lot, (*b*) measuring the homogeneity of large lots after mechanical mixing has occurred, (*c*) measuring the accuracy and uniformity of separation obtained with an air-blast seed separator for the analysis of replicate samples of seed, and (*d*) determining the maximum differences that may be expected between replicate samples of seed tested for germination.

These applications of statistical methods to the practice of seed testing have not only illustrated the importance and value of developing more standard and uniform procedures but have led to the adoption of standardized methods. In addition they have contributed to more effective and equitable enforcement of seed laws.

More recently Leggatt (255) has applied the principles of "Sequential Analysis" to the problems of seed testing. The advantages of this type of analysis are considered to be that the "quality of a product can be maintained with approximately one-half the sampling that is necessary by other methods of quality control or, alternatively, that with the same amount of sampling the degree of certainty or quality control may be increased or we may say that the limits of variability may be reduced". The procedure of Sequential Analysis, as described, "is to draw a number of samples and test them sequentially one after another basing a decision as to whether the lot is acceptable or unacceptable, or whether further testing is required, upon the total quantity of material analyzed up to that particular sample". He prepared a series of tables to be used in determining the proper decision in connection with counts from weed seeds in seed lots. The use of this principle of analysis requires the establishment of the "Acceptable Quality

Limit" and the "Unacceptable Quality Limit". It is probable, as suggested by Leggatt, that the application of "Sequential Analysis" to the science of seed testing will greatly aid in reducing the pressure of work and the size of test in relation to accuracy of results obtained.

ACKNOWLEDGMENTS

The major part of this paper was written during the fiscal year of 1946–47 when the writer was serving with the Food Supply Division of the Institute of Inter-American Affairs at Ascuncion, Paraguay, where library reference facilities are very limited. This limitation was largely overcome by the valuable assistance of former associates in the Iowa State College Seed Laboratory, namely, Dr. Duane Isley, Louis Bass, Mary Durrell, Frances Meehan and June Carlson, to whom the thanks of the writer are gratefully extended. Without their generous assistance in the preparation of citation summaries and that of Phyllis M. Porter, who did most of the typing, the completion of this paper would have been impossible. The long delay in preparation of the paper was unavoidable, largely because of the exigencies of the recent war. The writer must accept full responsibility for any errors or omissions of important references.

Since proofreading of this article in August, 1947, while the writer was on leave of absence in the United States before returning to Brazil, a number of papers have appeared to which reference might well be made. To do so would necessitate rewriting a portion of the article, which, of course, can not very well be done. Three papers of interest and related to two phases discussed in the article are *a*) Distinguishing the species of *Brassica* by their seed. Albina F. Musil, U. S. Dept. Agr., Misc. Publ. 63. 1948; and (*b*) the papers on seed treatment by R. W. Leukel and J. C. Walker in recent issues of THE BOTANICAL REVIEW.

LITERATURE CITED

1. Aberg, Ewert, Porter, R. H. and Robbins, Wayne A. Further experiments with the Iowa Air Blast Seed Separator for the analysis of small-seeded grasses. Ia. Agr. Exp. Sta. Res. Bull. 340. 1945.
2. ———— and Wiebe, G. A. Classification of barley varieties grown in the United States and Canada in 1945. U.S. Dept. Agr. Tech Bull. 907. 1946.
3. Afanasiev, Michel. A physiological study of dormancy in seed of *Magnolia acuminata*. N. Y. (Cornell) Agr. Exp. Sta., Mem. 208. 1937.

4. ————. Germinating *Nandina domestica seedlings.* Am. Nurseryman **78:** 5–6. 1943.
5. AKAMINE, ERNEST K. Methods of increasing the germination of koa haole seed. Hawaii Agr. Exp. Sta., Ciro. 21. 1942.
6. ————. The effect of temperature and humidity on viability of stored seeds in Hawaii. Hawaii Agr. Exp. Sta., Bull. 90. 1943.
7. ALCOCK, N. L. Notes on common diseases sometimes seed-borne. Trans. Bot. Soc. Edinb. **30:** 332–337. 1931.
8. ANDERSON, ALICE M. Germination of freshly harvested seed of Kentucky blue-grass. Proc. Assoc. Off. Seed Anal. **33:** 96–98. 1941.
9. ————. The effect of different temperatures on the germination of freshly harvested and mature seed of *Axonopus compressus.* Proc. Assoc. Off. Seed Anal. **33:** 99–102. 1941.
10. ————. The effect of light, temperature and potassium nitrate on the germination of *Agrostis tenuis* Sibth. and *A. tenuis* var. highland seed. Proc. Assoc. Off. Seed Anal. **36:** 112–125. 1946.
11. ANDRUS, C. F. Seed transmission of *Macrophoma phaseoli.* Phytopath. **28:** 620–634. 1938.
12. ANONYMOUS. Duration of viability in seeds. Gard. Chron. (London) **111:** 234. 1942.
13. ————. Photographs of normal and abnormal seedlings of various crop plants. U. S. Dept. Agr., Prod. & Mark. Adm., Grain Branch.
14. ARK, P. O. and LEACH, L. D. Seed transmission of bacterial blight of sugar beet. Phytopath. **36:** 549–553. 1946.
15. AXENTJEV, B. N. Über die Rolle der Schalen von Samen und Früchten, die bei der Keimung auf Licht reagieren. Beih. Bot. Centralbl. **46:** 119–202. 1930.
16. BAKER, K. F. and LOCKE, W. F. Perithecia of *Erisyphe cichoracearum* on *Zinnia* seed. Phytopath. **36:** 379–380. 1946.
17. BALDWIN, HENRY I. Catalase activity as a measure of viability of tree seeds. Am. Jour. Bot. **22:** 635–644. 1935.
18. ————. Forest tree seed. 1942.
19. BARTHOLOMEW, R. P. Seed treatment with plant hormones in crop production. Ark. Agr. Exp. Sta., Bull. 444. 1944.
20. BARTON, LELA V. Germination and storage of *Delphinium* seed. Bull. Am. Delphinium Soc. **2:** 12–14. 1933.
21. ————. Dormancy in *Tilia* seeds. Contr. Boyce Thompson Inst. **6:** 69–89. 1934.
22. ————. Storage of some coniferous seeds. Contr. Boyce Thompson Inst. **7:** 379–404. 1935.
23. ————. Storage of vegetable seeds. Contr. Boyce Thompson Inst. **7:** 323–334. 1935.
24. ————. A further report on the storage of vegetable seeds. Contr. Boyce Thompson Inst. **10:** 205–220. 1939.
25. ————. Storage of elm seeds. Contr. Boyce Thompson Inst. **10:** 221–233. 1939.
26. ————. Storage of some flower seeds. Contr. Boyce Thompson Inst. **10:** 399–427. 1939.
27. ————. Some effects of treatment of non-dormant seeds with certain growth substances. Contr. Boyce Thompson Inst. **11:** 181–205. 1940.
28. ————. Relation of certain air temperatures and humidities to viability of seeds. Contr. Boyce Thompson Inst. **12:** 85–102. 1941.
29. ————. Effect of moisture fluctuations on the viability of seeds in storage. Contr. Boyce Thompson Inst. **13:** 35–45. 1943.
30. ————. The storage of citrus seeds. Contr. Boyce Thompson Inst. **13:** 47–55. 1943.

31. ————. Some seeds showing special dormancy. Contr. Boyce Thompson Inst. 13: 259–271. 1944.

32. ————. A note on the viability of seeds of maga, *Montezuma speciossima*. Contr. Boyce Thompson Inst. 13: 423–426. 1945.

33. ————. Viability of seeds of *Fraxinus* after storage. Contr. Boyce Thompson Inst. 13: 427–432. 1935.

34. ————. Respiration and germination studies of seeds in moist storage. Ann. N. Y. Acad. Sci. 46: 185–208. 1945.

35. ———— and Garman, Helen R. Effect of age and storage conditions of seeds on the yields of certain plants. Contr. Boyce Thompson Inst. 14: 243–255. 1946.

36. ———— and Schroeder, Eltora M. Dormancy in seeds of *Convallaria majalis* L. and *Smilacina racemosa* (L.) Desf. Contr. Boyce Thompson Inst. 12: 277–300. 1942.

37. Bartsinskii, R. M. O prorastanii semian zarazikhi, porazhaiuschei podsolnechnik. Doklady Akademii Nauk USSR. 1: 340–346. 1934.

38. Beattie, J. H. and Boswell, Victor R. Longevity of onion seed in relation to storage conditions. U. S. Dept. Agr. Circ. 512. 1939.

39. ————, Jackson, A. M. and Currin, R. E. Effect of cold storage and age of seed on germination and yield of peanuts. U. S. Dept. Agr. Circ. 233. 1932.

40. Beery, Jerald. Seasonal maturity, dormancy and germination of seeds of bluegrass, timothy and oats. Unpub. Thess, Ia. State Coll. Lib. 1944.

41. Bennedict, H. M. and Robinson, J. Studies on the germination of guayule seed. U. S. Dept. Agr. Tech. Bull. 921. 1946.

42. Berkeley, G. H. and Madden, G. O. Transmission of streak and mosaic diseases of tomato through seed. Sci. Agr. 13: 455–457. 1933.

43. Bishop, L. R. Second memorandum on barley germination. Jour. Inst. Brewing 51: 215. 1945.

44. Bond, Lora. Colchicine stimulation of seed germination in *Petunia axillaris*. Jour. Hered. 33: 200–201. 1942.

45. Bondartzeva-Monteverde, Mme. V. N. and Vassilievsky, N. I. [Ascochytosis of the pea]. Acad. Sci. Press. Moscow, USSR; Rev. Appl. Myc. 17: 427–428. 1938. [Russian; English summary].

46. Borthwick, H. A. Thresher injury in baby Lima bean. Jour. Agr. Res. 44: 503–510. 1932.

47. ————. Retarded germination in the seed of *Hypericum perforatum* caused by calcium. Bot. Gaz. 98: 270–282. 1936.

48. Boswell, Victor, Toole, E. H., Toole, Vivian K. and Fisher, D. F. A study of rapid deterioration of vegetable seeds and methods for its prevention. U. S. Dept. Agr. Tech. Bull. 708. 1940.

49. Boyd, O. C. Evidence of the seed-borne nature of late blight of tomatoes. Phytopath. 25: 7. 1935.

50. Bressman, E. N. and Barss, H. P. Experiments with head smut of corn in western Oregon. Phytopath. 23: 396–403. 1933.

51. Brison, F. R. Influence of storage conditions upon the germination of onion seed. Proc. & Trans. Texas Acad. Sci. 25: 69–71. (1941) 1942.

52. Brown, E. Development of seed testing. U. S. Dept. Agr., Bur. Pl. Ind., Div. Cer. Crops & Dis; Mim. Circ. 1939.

53. ———— and Toole, E. H. The evaluation of seed tests. Proc. Int. Seed Test. Assoc. 3: 203–206. 1931.

54. Brown, E. O. and Porter, R. H. An improved method of testing seeds of Kentucky bluegrass (*Poa pratensis* L.). Proc. Assoc. Off. Seed. Anal. 44–49. 1935.

55. ———— and ————. The viability and germination of seeds of *Convolvulus arvensis* L. and other perennial weeds. Ia. Agr. Exp. Sta., Res. Bull. **294**: 475–504. 1942.

56. Brown, James W. Respiration of acorns as related to temperature and after-ripening. Pl. Physiol. **14**: 621–645. 1939.

57. Brown, R. and Edwards, M. Effects of thiourea and allylthiourea on the germination of the seed of *Striga lutea*. Nature **155**: 455–456. 1945.

58. Burunjik, May. The conditions necessary for the germination of the seeds of some plants native to the Chicago region. Diss., Univ. Chicago. 1933.

59. Busse, W. F. Effect of low temperatures on the germination of seed. Bot. Gaz. **89**: 169–179. 1930.

60. Bychickhina, E. A. [Winter wheats ot Ukraine and their after-ripening]. [Russian]. Bull. Appl. Bot., Genet. & Pl. Breed. **23**: 299–347. 1930.

61. Calvert, E. L. and Muskett, A. E. Blind seed disease of ryegrass. Nature **153**: 287–288. 1944.

62. Chabrolin, C. H. La germination des graines de *Thesium humile* exige l'intervention de champignons saprophytes. Comt. Rend. Acad. Sci. (Paris) **199**: 225–226. 1934.

63. Chadwick, L. C. and Swartley, John C. Further studies on the effects of synthetic growth substances on cuttings and seeds. Proc. Am. Soc. Hort. Sci. **38**: 690–694. 1940.

64. Chelmar, F. Possibilities of accelerating seed analysis and the determination of variety by employing luminescence tests in ultra-violet light. Proc. Int. Seed Test. Assoc. **6**: 435–445. 1934.

65. Chester, K. Starr. Gravity grading, a method for reducing seed-borne disease in cotton. Phytopath. **28**: 745–749. 1938.

66. Chilton, S. J. P. The occurrence of *Helminthosporium turcicum* in the seeds and glumes of Sudan grass. Phytopath. **30**: 533–536. 1940.

67. ————. Some pathogenic fungi occurring in the seed of red and subterranean clover. Phytopath. **32**: 738–739. 1942.

68. Chippindale, H. G. The effect of soaking in water on the "seeds" of *Dactylis glomerata* L. Ann. Bot. **47**: 841–849. 1933.

69. Christoff, Alexander. [The *Pleospora* disease of the cultivated poppy]. Min. Agr. Nat. Domains, Sofia. 1930. [English summary].

70. Clark, J. Allen and Bayles, B. B. Classification of wheat varieties grown in the United States in 1939. U. S. Dept. Agr., Tech. Bull. 795. 1942.

71. Coe, F. M. and Gerber, Robert K. Preliminary studies of the after-ripening and germination of cherry seeds. Proc. Utah. Acad. Sci., Arts & Let. **11**: 185–187. 1934.

72. Coffman, F. A. and Stanton, T. R. Dormancy in fatuoid oat kernels. Jour. Am. Soc. Agron. **32**: 459–466. 1940.

73. ———— and ————. Variability in germination of freshly harvested *Avena*. Jour. Agr. Res. **57**: 57–72. 1936.

74. ———— and Taylor, J. W. Widespread occurrence and origin of fatuoids in Fulghum oats. Jour. Agr. Res. **52**: 123–131. 1936.

75. Contributions to the Handbook on Seed Testing prepared by the Assoc. Off. Seed. Anal Wright, W. H.
    The seeds of *Silene* and *Lychnis*.
    The seeds of perennial ryegrass and meadow fescue.
    The seeds of *Sonchus*.
    The seeds of the genus *Poa* commonly found on the market.
    Millets.

76. Cook, H. T. Occurrence of oospores of *Peronospora effusa* with commercial spinach seed. Phytopath. **25**: 11–12. 1935.

77. Corkill, L. Inheritance of fluorescence in ryegrass. Nature **130**: 134. 1932.

78. Cormock, M. W. Studies on *Ascochyta imperfecta*, a seed- and soil-borne parasite of alfalfa. Phytopath. **35**: 838–855. 1945.

79. Coukos, C. J. Seed dormancy and germination in some native grasses. Jour. Am. Soc. Agron. **36**: 337–345. 1944.

80. Cox, L. C. Munger, H. M. and Smith, E. A. A germination inhibitor in the seed coats of certain varieties of cabbage. Pl. Physiol. **20**: 289–294. 1945.

81. Crescini, Francesco. Forme di Canapa. III. La Germin abilita. Nuovo Gior. Bot. Ital. **50**: 209–231. 1943.

82. Crocker, William. Life span of seeds. Bot. Rev. **4**: 235–274. 1938.

83. ———— and Barton, Lela V. After-ripening, germination and storage of certain rosaceous seeds. Contr. Boyce Thompson Inst. **3**: 385–404. 1931.

84. Crosier, Willard F. Detection and identification of seed-borne organisms. Proc. Assoc. Off. Seed Anal. **27**: 87–92. 1935.

85. ————. Prevalence and significance of fungous associates of pea seeds. Proc. Assoc. Off. Seed Anal. **28**: 101–107. 1936.

86. ————. Abnormal germination of wheat caused by organic mercurials. Proc Assoc. Off. Seed Anal. **23/26**: 284. 1930/33. (1938).

87. ————. The planting value of oats and barley collected from farmers' drills and granaries. N. Y. (Geneva) Agr. Exp. Sta., Bull. 681. 1938.

88. ————. Materials and methods in controlling seed-contaminating micro-organisms. Proc Assoc. Off. Seed Anal. **34**: 104–108. 1942. (1943).

89. ————. Some fungi found in seed stocks during recent years. Proc. Assoc. Off. Seed Anal. **35**: 99-102. 1943–1944.

90. ————. Chemical control of seed-borne fungi during germination testing of peas and sweet corn. Phytopath. **36**: 92–99. 1946.

91. ———— and Nelson, C. Chemical control of molds when germinating lima beans. Proc. Assoc. Off. Seed Anal. **30**: 189–194. 1938.

92. ———— and Patrick, Stewart. Influence of chemical and thermal treatments on infection of cruiferous seedlings by *Alternaria* spp. and *Rhizopus nigricans*. Proc. Assoc. Off. Seed Anal. **31**: 116–120. 1940.

93. ———— and ————. Arasan for control of fungi in germinating corn seed. Phytopath. **36**: 162–164. 1946.

94. ———— and Weimer, Dolores. Some fungi associated with grass seed. Proc. Assoc. Off. Seed Anal. **31**: 120–14. 1940.

95. Darlington, H. T. The sixty-year period for Dr. Beal's seed viability experiment. Am. Jour. Bot. **28**: 271–273. 1941.

96. Davidson, W. A. Seedling identification. Proc. Assoc. Off. Seed Anal. **27**: 40–43. 1935.

97. Davies, P. A. Effect of high pressure on germination of seeds of *Medicago sativa* and *Melilotus alba*. Jour. Gen. Physiol. **9**: 805–809. 192.

98. Davies, W., Atkins, G. A. and Hudson, P. C. B. The effect of ascorbic acid and certain indole derivatives on the regeneration and germination of plants. Ann. Bot. **1**: 329–351. 1937.

99. Davis, G. N. Laboratory detection of smut in oats. Proc. Assoc. Off. Seed Anal. **27**: 93. 1935.

100. ————. Germination of cucurbit seed in sand. Proc. Assoc. Off. Seed Anal. **29**: 133–135. 1937.

101. ————. Germination of treated and untreated pea seeds in autoclaved and unautoclaved soil. Proc. Assoc. Off. Seed Anal. **29**: 121–125. 1937.

102. Davis, W. C. Phenolase activity in relation to seed viability. Pl. Physiol. 6: 127–138. 1931.

103. Davis, Wilmer E. The use of catalase as a means of determining the viability of seeds. Proc. Assoc. Off. Seed Anal. 18: 33–39. 1926.

104. ————. The development of dormancy in seeds of cokle-bur (*Xanthium*). Am. Jour. Bot. 17: 77–87. 1930.

105. ————. Primary dormancy, after-ripening and the development of secondary dormancy in embryos of *Ambrosia trifida*. Am. Jour. Bot. 17: 58–76. 1930.

106. Delizo, Teodora. Methods of hastening the germination of the seeds of akle. Philippine Jour. For. 1: 99–102. 1938.

107. Deming, G. W. and Robertson, D. W. Dormancy in small grain seeds. Col. Agr. Exp. Sta., Tech. Bull. 5: 3–12. 1933.

108. Dexter, S. T. Commercial hormone dusts for seed treatment: a second report. Mich. Agr. Exp. Sta., Quart. Bull. 25: (4): 279–282. 1943.

109. Dillman, A. C. and Toole, E. H. Effect of age, condition and temperature on the germination of flaxseed. Jour. Am. Soc. Agron. 29: 23–29. 1937.

110. Dinnis, E. R. and Jordon, S. The germination of freshly harvested and of stored seeds of sea pea. Jour. So.-Eastern Agr. Coll. (Kent, England) 44: 140–142. 1939.

111. Doak, B. W. A chemical method for determination of type of white clover. New Zeal. Jour. Agr. & Tech. 14: 359–365. 1933.

112. Dorchester, C. S. Seed and seedling characters in certain varieties of soybeans. Jour. Am. Soc. Agron. 37: 223–232. 1945.

113. Doroshenko, A. V. Plasmolytic method of determining the germinative capacity of seeds. Bull. Appl. Bot., Genet. & Pl. Breed. IV. 2: 119. 1937.

114. Dorph-Petersen, K. Examination of ryegrass (*Lolium* sp.) in ultraviolet light made at the Danish State Seed Testing Sta. Proc. Int. Test. Assoc. 6: 446–452. 1934.

115. Doyer, L. C. Iets over den Gezondheidstoestand der Zaaizaden en Verschillende Jaren. Tijds. Plantenziekten 36: 65–74. 1930.

116. ————. De Gezondheidstoestand van Klaverzaad, in verband met de Kewing van dit zaad en de invloed van ontsmetting op dezen toestand. Tijds. Plantenziekten 40: 54–61. 1934.

117. ————. Manual for the determination of seed-borne organisms. 59 pp. 1938.

118. ————. The importance of seed as a conveyer of vegetative diseases and pests. Tejds. Fl. Nick 47: 14–24. 1941.

119. Drake, Vera. Case against baldhead plants. Seed World 56: 13. 1943.

120. ————. Comparison of yield in snap beans between normal and certain types of abnormal plants. Proc. Assoc. Off. Seed Anal. 36: 164–172. 1946.

121. Dungan, George H. Influence of age on the value of seed corn. Trans. Ill. Acad. Sci. 33: 28–29. 1940.

122. ———— and Koehler, Benjamin. Age of seed corn in relation to infection and yielding capacity. Jour. Am. Soc. Agron. 36: 436–443. 1944.

123. Dunlap, A. A. Seedling culture in sand to prevent damping off. Phytopath. 26: 278–284. 1936.

124. ———— and McDonnell, A. D. Testing germination in sand. Jour. For. 37: 330–332. 1939.

125. Dunn, L. E. Influence of low temperature treatments on the germination of seeds of sweet clover and smooth vetch. Jour. Am. Soc. Agron. 31: 687–694. 1939.

126. Edwards, Thomas The germination and growth of *Peltandra virginica* in the absence of oxygen. Bull. Torrey Bot. Club **60**: 573–581. 1933.

127. Eggebrecht, H. and Bethmann, W. Das Selenfärbeverfahren im Vergleich zu der üblichen Keimprüfung insbesondere bei Wintergerste mit Keimruhe. Ang. Bot. **21**: 448–455. 1939.

128. Eggers, E. R. Effect of the removal of the seed coats on avocado seed germination. Cal. Avocado Soc., Yr. Bk. **1942**: 41–43. 1942.

129. Eidmann, Franz Erich. Eine neue biochemische Methode zur Erkennung des Aussaatwertes von Samen. Proc. Int. Seed Test. Assoc. **10**: 203–209. 1938.

130. Eliason, E. J. and Heit, C. E. The results of laboratory tests as applied to large scale extraction of red pine seed. Jour. For. **38**: 426–429. 1940.

131. Erickson, E. L. The South Dakota seed blower. Proc. Assoc. Off. Seed Anal. **35**: 91–95. 1944.

132. ———— and Porter, R. H. Laboratory tests of field crop seeds as indicators of seedling value. Ia. Agr. Exp. Sta., Res. Bull. **239**: 275–213. 1938.

133. Etheridge, W. C., Helm, C. A. and King, B. M. A classification of soybeans. Mo. Agr. Exp. Sta., Bull. 13.

134. Fischer, George W. The blind-seed disease of ryegrass (*Lolium* spp.) in Oregon. Phytopath. **34**: 934–935. 1944. [Abstract].

135. Flemion, Florence Physiological and chemical changes preceding and during the after-ripening of *Symphoricarpus racemosus* seeds. Contr. Boyce Thompson Inst. **6**: 91–102. 1934.

136. ————. A rapid method for determining the germinative power of peach seed. Contr. Boyce Thompson Inst. **8**: 289–293. 1936.

137. ————. Breaking the dormancy of seeds of *Crataegus* species. Contr. Boyce Thompson Inst. **9**: 409–423. 1938.

138. ————. A rapid method for determining the viability of dormant seeds. Contr. Boyce Thompson Inst. **9**: 339–351. 1938.

139. ————. Further studies on the rapid determination of the germinative capacity of seeds. Contr. Boyce Thompson Inst. **11**: 455–464. 1941.

140. ———— and Parker, Elinor. Germination studies of seeds of *Symphoricarpus orbiculatus*. Contr. Boyce Thompson Inst. **12**: 301–307. 1942.

141. ———— and Uhlmann, Gloria. Further studies of embryoless seeds in the Umbelliferae. Contr. Boyce Thompson Inst. **14**: 283–293. 1946.

142. Flyaksberger, K. A., Antropov, V. I., Antropova, V. P., Bakhtev, K. H. and Mordvinkina, A. I. Opredelitel Nastoyastchikh Khlebov. 4th ed. 1939.

143. Fraser, J. G. C. and Gfeller, F. Two new methods of distinguishing certain Canadian wheats. Sci. Agr. **15**: 564–572. 1935.

144. ———— and ————. The inheritance and use of phenol colour reaction in hard red spring wheats. Sci. Agr. **17**: 243–249. 1936.

145. Garman, Helen R. and Barton, Lela V. Response of lettuce seeds to thiourea treatments as affected by variety and age. Contr. Boyce Thompson Inst. **14**: 229–241. 1946.

146. ———— and ————. Germination of seeds of *Panicum anceps* Michx. Contr. Boyce Thompson Inst. **14**: 117–122. 1946.

147. Geddes, W. F. Factors affecting the keeping quality of grain in storage. Am. Miller **71**: 40, 93. 1943.

148. Gentner, G. Ueber die Vervendbarkheit von ultravioletten Strahlen bei der Samenprüfung. Practische Blätter f. Pflanzenbau u. Pflanzelschutz **6**: 166–172. 1929.

149. Giersbach, Johanna. After-ripening and germination of cottoneaster seeds. Contr. Boyce Thompson Inst. **6:** 323–338. 1934.

150. Gordon, W. L. The occurrence of *Fusarium* species in Canada. I. Species of *Fusarium* isolated from samples of cereal seed in Manitoba. Canad. Jour. Res., C **22:** 282–286. 1944.

151. Gorman, M. J. and Lafferty, H. A. A method of distinguishing the seedlings of swedish turnip (*Brassica napus* L. var. *napobrassica* (L.) Reichb.) from those of rape (*B. napus* L. var. *biennis* (Schull. et Mart.) Reichb.). Sci. Proc. Royal Dublin Soc. **20:** 119–124. 1931.

152. Grace, N. H. Effect of phytohormones on seeds damaged by formaldehyde and other disinfectants. Canad. Jour. Res., C, **16:** 313–329. 1938.

153. Gray, Elizabeth G. *Phialea mucosa* sp. nov., the blind-seed fungus. Trans. Brit. Myc. Soc. **25:** 329–333. 1942.

154. Greaney, F. J. and Machacek, J. E. The prevalence and control of seed-borne diseases of cereals in Manitoba. Sci. Agr. **26:** 59–78. 1946.

155. Griffiths, A. E. The viability of lettuce seed: A physiological and microchemical study. N. Y. (Cornell) Agr. Exp. Sta. Mem. 245. 1942.

156. Griswold, Sylvia M. Effect of alternate moistening and drying on germination of western range plants. Bot. Gaz. **98:** 243–269. 1936.

157. Groves, J. W. and Skolko, A. J. Notes on seed-borne fungi. I. *Stemphylium*. Canad. Jour. Res., C, **22:** 190–199. 1944.

158. ————. Notes on seed-borne fungi. II. *Alternaria*. Canad. Jour. Res., C, **22:** 217–234. 1944.

159. ————. Notes on seed-borne fungi. III. *Curvularia*. Canad. Jour. Res., C, **23:** 94–104. 1945.

160. ————. Notes on seed-borne fungi. IV. *Acremoniella, Chalmydomyces* and *Trichocladium*. Canad. Jour. Res., C, **24:** 74–80. 1946.

161. Guillaumin, A. Le maintien des graines dans un milien privé d'oxygène comme moyen de prolonger leur faculté germinative. Compt. Rend. Acad. Sci. (Paris) **187:** 571–572. 1928.

162. Gurewitach, Alexander. Über eine Methode zur Bestimmung der Keimfähigkeit ohne Keimprüfung. Ber. Deut. Bot. Gaz. **53:** 303–318. 1935.

163. Hamid, M. Abdul. Longevity of cotton seed delinted with sulphuric acid. Emp. Cotton Growing Rev. **15:** 312–314. 1938.

164. Hamly, Douglas H. Softening the seeds of *Melilotus alba*. Bot. Gaz. **93:** 345–375. 1932.

165. Hao, Kin-shen. Über Saatgutprüfung auf biochemischem Vege. Zeits. Forst. u. Jagdw. **71:** 141–156, 187–204, 249–269. 1939.

166. Harding, J. H. Acacia germination trials. Australian Forestry **5:** 53–56. 1940.

167. Hare, J. F. and King, C. J. The winter carry-over of angular leaf spot infection in Arizona cotton fields. Phytopath. **30:** 679–684. 1940.

168. Harter, L. L. Thresher injury a cause of bald-head in beans. Jour. Agr. Res. **40:** 371–384. 1930.

169. Hasegawa, Kozo. On the determination of vitality in seed by reagents. Proc. Int. Seed Test. Assoc. **7:** 148–153. 1935.

170. ————. On a method of determining seed vitality by a certain reagent. Jap. Jour. Bot. **8:** 1–4. 1936.

171. Haut, I. C. Catalase activity in relation to the after-ripening of fruit tree seeds. Proc. Am. Soc. Hort. Sci. **29:** 375–379. 1932.

172. Heit, C. E. Laboratory germination of belladonna (*Atropa belladonna*) seed. Proc. Assoc. Off. Seed Anal. **33:** 84–87. 1941.

173. ———. Laboratory germination results with certain flower seeds. Proc. Assoc. Off. Seed. Anal. **36**: 141–150. 1946.

174. ———. Summarized laboratory germination data on over 100 flower seed species. Assoc. Off. Seed. Anal. News letter **20**: 13–16. 1946.

175. ——— and Eliason, E. J. Coniferous tree seed testing and factors affecting germination and seed quality. N. Y. (Geneva) Agr. Exp. Sta., Tech. Bull. 255. 1940.

176. ——— and Nelson, Carrie. Approximate germination tests of dormant seeds by excising embryos. Proc. Assoc. Off. Seed. Anal. **33**: 87–89. 1941.

177. Helgeson, Earl A. Impermeability in mature and immature sweet-clover seeds as affected by conditions of storage. Trans. Wis. Acad. Sci., Arts & Lett. **27**: 193–206. 1932.

178. Hellbo, E. A study of morphological characters on the young plant of some cruciferous species grown under artificial light. Meddelanden fran Statens Centrala Frokontrollanstalt. N.r. 6–10. 1931-1935.

179. Henderson, W. J. Yellow dwarf a virus disease of onion, and its control. Ia. Agr. Exp. Sta., Res. Bull. 188. 1928.

180. Henry, A. W. and Campbell, J. A. Inactivation of seed-borne plant pathogens in the soil. Canad. Jour. Res., C, **16**: 331–338. 1938.

181. Henry, Helen H. The seeds of quack grass and certain wheat grasses compared. Jour. Agr. Res. **35**: 537–546. 1927.

182. Hillman, F. H. Identification of species and varieties of *Agrostis* with plates illustrating distinguishing differences. U.S. Dept. Agr. Bur. Pl. Ind. [Unnumbered separate.]

183. ——— and Henry, Helen H. The more important forage-plant seeds and incidental seeds commonly found with them. 15 plates of photographs of drawings of seeds. U.S. Dept. Agr., Bur Pl. Ind., Div. Seed Invest. Revised. 1935.

184. Hills, K. Loftus. Dormancy and hardseededness in *Trifolium subterraneum*. II. The progress of after-harvest ripening. Jour. Australia Coun. Sci. & Ind. Res. **17**: 186–190. 1944.

185. ———. Dormancy and hardseededness in *Trifolium subterraneum*. III. The effect upon dormancy of germination at three different constant temperatures. Jour. Australia Coun. Sci. & Ind. Res. **17**: 191–196. 1944.

186. ———. Dormancy and hardseededness in *Trifolium subterraneum*. IV, V, VI. Jour. Australia Coun. Sci. Ind. Res. **17**: 242–257. 1944.

187. Hollowell, E. A. The pubescent characteristics of red clover, *Trifolium pratense*, as related to the determination of the origin of the seed. Jour. Am. Soc. Agron. **32**: 1–11. 1940.

188. Hope, Claude, Stoutmeyer, V. T. and Close, A. W. The control of damping-off by the use of sphagnum for seed germination. Proc. Am. Soc. Hort. Sci. **39**: 397–406. 1941.

189. Horn, Claud L. and Natal Colon, Jose E. Acid scarification of the seed of two Cuban fiber plants. Jour. Am. Soc. Agron. **34**: 1137–1138. 1942.

190. Horsfall, J. G. Red oxide of copper as a dust fungicide for combatting damping-off by seed treatment. N. Y. Agr. Exp. Sta., Bull. (Geneva) 615. 1932.

191. ———. Combating damping-off. N. Y. (Geneva) Agr. Exp. Sta., Bull 683. 1938.

192. Hull, Fred H. Inheritance of rest period of seeds and certain other characters in the peanut. Fla. Agr. Exp. Sta., Bull. 314. 1937.

193. Humphries, W. R. and Hurst, W. M. Moisture changes in some agricultural products due to atmospheric conditions. Agr. Eng. **16**: 8–12. 1935.

194. Hutchings, Selar S. Light in relation to the seed germination of *Mimulus ringens* L. Am. Jour. Bot. **19:** 632–643. 1932.

195. Hutton, Mary Erne-Jean and Porter, R. H. Seed impermeability and viability of native and introduced species of Leguminosae. Ia. State Coll. Jour. Sci. **12:** 5–24. 1937.

196. Hwang, Y. and Pearse, H. L. The response of seeds and seedlings to treatment with indolylacetic acid. Am. Bot. **4:** 31–37. 1940.

197. Hyde, E. O. C. Detecting *Pullularia* infection in ryegrass seed crops. New Zeal. Agr. **42:** 301–302. 1938.

198. Ingalls, R. A. A study of the occurrence of baldheads in beans and a comparison of their field performance with that of normal plants. Proc. Assoc. Off. Seed Anal. **36:** 177–184. 1946.

199. Isaac, Leo A. Cold storage prolongs the life of noble fir seed and apparently increases germination power. Ecology **15:** 216–217. 1934.

200. Isely, Duane. A study of conditions that affect the germination of *Scirpus* seeds. N. Y. (Cornell) Agr. Exp. Sta., Mem. 257. 1944.

201. ————. Investigations in seed classification by family characters. Ia. Agr. Exp. Sta., Res. Bull. 351. 1947.

202. ————. Seed characters of alfalfa and certain other species of *Medicago*. Ia. State Coll. Jour. Sci. **21:** 153–159. 1947.

203. ————. Seeds of weedy thistles. Proc. Assoc. Off. Seed Anal. **37:** [In press.] 1947.

204. Johnson, L. P. V. General preliminary studies on the physiology of delayed germination in *Avena fatua*. Canad. Jour. Res., C, **13:** 283-300. 1935.

205. ————. The inheritance of delayed germination in hybrids of *Avena fatua* and *A.* (*sativa*) Canad. Jour. Res., C, **13:** 367–387. 1935.

206. ————. Effect of chemical treatments on the germination of forest tree seeds. For. Chron. **22:** 17–24. 1946.

207. Jones, F. R. Seed borne diseases of sweet clover. Phytopath. **28:** 661–662. 1938.

208. ————. Four fungus parasites of sweet clover infecting seed. Phytopath. **29:** 912–913. 1939.

209. Jones, J. P. A physiological study of dormancy in vetch seed. N. Y. (Cornell) Agr. Exp. Sta., Mem 120. 1928.

210. Jorgensen, C. A. Nogle Undergelser over Plantesygdomme med Frosmitte. Tidsskr. Planteavl. **40:** 119–147. 1934.

211. Justice, Oren L. A study of dormancy in seeds of *Polygonum*. N. Y. (Cornell) Agr. Exp. Sta., Mem. 235. 1941.

212. ————. Viability and dormancy in seeds of *Polygonum amphibium* L., *P. coccineum* Muhl. and *P. hydropiperoides* Michx. Am. Jour. Bot. **31:** 369–377. 1944.

213. ————. Germination of seed of Florida beggarweed. Proc. Assoc. Off. Seed Anal. **34:** 103–104. 1944.

214. ————. A review of literature on the use of the fluroescence test for the classification of *Lolium* species and hybrids. Proc. Assoc. Off. Seed Anal. **36:** 86–93. 1946.

215. ———— and Marks, Regna W. Germination of unscarified and scarified seed of *Lathyrus hirsutus* L., under laboratory, greenhouse and field conditions. Proc. Assoc. Off. Seed Anal. **35:** 104–114. 1944.

216. ———— and Whitehead, M. D. Viability of velvet bean, *Stizolobium* spp., seed as affected by date of harvest, weathering, storage and lodging Jour. Am. Soc. Agron. **34:** 1000–1009. 1942.

217. ———— and ————. Viability of bulblets of *Allium canadense* and *A. vineale* occurring in seeds of cereals and crimson clover. Proc. Assoc. Off. Seed Anal. **34:** 109–114. 1942.

218. ———— and ————. Seed production, viability and dormancy in the nut grasses, *Cyperus rofundus* and *Cyperus esculentus.* Jour. Agr. Res. **73:** 303–318. 1946.
219. KADOW, K. J. Seed transmission of *Verticillium* wilt of eggplant and tomato. Phytopath. **24:** 1265–1268. 1934.
220. KEARNS, VIVIAN and TOOLE, E. H. Relation of temperature and moisture content to longevity of Chewings fescue seed. U.S. Dept. Agr. Tech. Bull. 670. 1939.
221. ————. Temperature and other factors affecting the germination of fescue seed. U.S. Dept. Agr. Tech. Bull. 638. 1939.
222. KENDRICK, JAMES B. Fruit invasion and seed carriage of tomato fusarium wilt. Phytopath. **34:** 1005. 1944.
223. ————. Seed transmission of cowpea *Fusarium* wilt. Phytopath. **21:** 979–983. 1931.
224. ————. Seed transmission of *Fusarium* yellows of beans. Phytopath. **24:** 1139. 1934. [Abstract].
225. ———— and BAKER, KENNETH F. Bacterial blight of garden stocks and its control by hot water seed treatment. Cal. Agr. Exp. Sta., Bull. 665. 1942.
226. KINCAID, RANDALL R. The effects of certain environmental factors on germination of Florida cigar wrapper tobacco seeds. Fla. Agr. Exp. Sta., Bull 277. 1935.
227. ———— The effect of storage conditions on the viability of tobacco seed. Jour. Agr. Res. **67:** 407–410. 1943.
228. KIESSELBACH, T. A. Crop response to hormone seed treatment. Jour. Am. Soc. Agron. **35:** 321–331. 1943.
229. KOBLET, RUDOLF. Über die Kiemung von *Pinus strobus* unter besonderer Berücksichtigung der Herkunft des Samens. Ber. Schweiz. Bot. Geo. **41:** 199–283. 1932.
230. ————. Die Beeinflussung der Pflanzenwachstums durch Saatgutbehandlung. Sammelreferat. Landw. Jahrb. Schweiz **56:** 278–288. 1942.
231. KOEHLER, BENJAMIN. Effect of storage on yields of farm seed treated for disease control. Ill. Agr. Exp. Sta., Bull 476: 258–276. 1941.
232. ———— and DUNGAN, GEORGE H. Disease infection and field performance of bin and hanger dried seed corn. Jour. Am. Soc. Agron. **32:** 768–781. 1940.
233. KONDO, MANTARO, MATSUSHIMA, SHUZO, and OKAMURA, TAMOTSU. B-Vitamin der im kohlensauren und luftdichten Verschlusse 4 Jahre lang aufbewahrten Reiskorner. Proc. Imp. Acad. (Tokio) **5:** 159–160. 1929.
234. KROEGER, GARNETTE S. Dormancy in seeds of *Impatiens balsamina* L. Contr. Boyce Thompson Inst. **12:** 203–212. 1941.
235. KRZESKIEWICZ, VLODZIMIERA. Okreslanie potencjalnej zdolnosci kiekowania nasion sosny za pomoco metody barwienia [Keimpotenzbestimmung bei Kiefernsamen mittels des Indigokarminfäburgsverfahren.]. (German Summary). Rozprawy i Surawozdania. Inst. Badowzcy Lasow Panstwowych. Warsaw. A. **44:** 7-45. 1939.
236. LAFFERTY, H. A. The loss of vitality in stored farm seeds. Dept. Agr. Jour. (Dublin) **30:** 237–245. 1931.
237. LAKON, GEORG. Das Schwinden des Keimfähigheit der Samen insbesondere der Getreidefrüchte. Ber. Deut. Bot. Ges. **57:** 191–203. 1939.
238. ————. Topographischer Nachweis der Keimfähigkeit der Getreidefruchte durch Tetrazolumsalze. Ber. Deut. Bot. Ges. **60:** 299-305. 1942.
239. ————. Topographischer Nachweis der Keimfähigkeit von Mais durch Tetrazoliumsalze. Ber. Deut. Bot. Ges. **60:** 434-444. 1942.

240. Landau, N. The effect of hetero-auxin on the germination of some seeds. Palestine Jour. Bot. **1**: 409–412. 1940.

241. Laughland, J. and Laughland, D. H. The effect of age on the vitality of soybean seed. Sci. Agr. **20**: 236–237. 1939.

242. Leach, Lysle D. Downy mildew of the beet, caused by *Peronospora schachtii* Fuckel. Hilgardia **6**: 203–251. 1931.

243. ——— and Borthwick, H. A. Distribution of downy mildew mycelium in spinach fruits. Phytopath. **24**: 1021–1025. 1934.

244. ———. Growth rates of host and pathogen as factors determining the severity of pre-emergence damping off. Phytopath. **34**: 12. 1944. [Abstract].

245. ———and Smith, Paul C. Effect of seed treatment on protection, rate of emergence and growth of garden peas. Phytopath. **35**: 191–206. 1945.

246. Leggatt, C. W. The agricultural value of hard seeds of alfalfa and sweet clover under Alberta conditions. Sci. Agr. **8**: 243–266. 1927.

247. ———. Further studies on the hard seed problem: Alfalfa and sweet clover. Sci. Agr. **11**: 418–427. 1931.

248. ———. A further note on catalase activity as a measure of seed viability. Canad. Jour. Res., C, **9**: 571–573. 1933.

249. ———. Short note on a new blower. Proc. Assoc. Off. Seed Anal. **29**: 103–104. 1937.

250. ———. A new seed blower. Proc. Assoc. Off. Seed Anal. **30**: 120–132. 1938.

251. ———. Contributions to the study of statistics of seed testing. VIII. Further studies on the distribution of particles differing in specific gravity or size. Proc. Int. Seed Testing Assoc. **11**: 25–39. 1939.

252. ———. Statistical aspects of seed testing. Bot. Rev. **5**: 505–529. 1939.

253. ———. The "climax" blowing point in the testing of grass seed for percentages of pure live seeds. Contr. 657, Div. Bot. & Pl. Path., Sci. Serv., Dept. Agr. Ottawa, Canada. 1941.

254. ———. Germination of seeds of three species of *Agrostis*. Canad. Jour. Res., C, **24**: 7–21. 1946.

255. ———. Sequential analysis. Proc. Assoc. Off. Seed Anal. **36**: 76–84. 1946.

256. Lemmon, Paul E., Brown, Robert L. and Chapin, Wilbur E. Sulfuric acid treatment of beach pea, *Lathyrus maritimus* and silvery pea, *L. littoralis*, to increase germination, seedling establishment, and field stands. Jour. Am. Soc. Agron. **35**: 177–191. 1943.

257. Leukel, R. W. Chemical seed treatments for the control of certain diseases of sorghum. U.S. Dept. Agr., Tech. Bull. 849. 1943.

258. ——— and Martin, John H. Seed rot and seedling blight of sorghum. U.S. Dept. Agr., Tech. Bull. 839. 1943.

259. Levitt, J. and Hamm, P. C. A method of increasing the rate of seed germination of *Taraxacum kok-saghys*, Pl. Physiol. **18**: 288–293. 1943.

260. Linehan, P. A. and Mercer, S.P. Fluorescence of *Lolium* seedlings in ultra violet light. Nature **131**: 202–203. 1933.

261. Lowe, Alvin E. Viability of buffalo grass seed found in the walls of a sod house. Jour. Am. Soc. Agron. **32**: 891–893. 1940.

262. Ludlam, F. X. and Owen, C. H. Identification of barley varieties grown in Canada by examination of threshed grain. Wallerstein Lab. Comm. **8**: 179–193. 1945.

263. Ludtke, Max and Achmed, Hikmet. Über einen pflanzlichen Welkstoff. Biochem. Zeits. **257**: 256–266. 1933.

264. Lute, Anna M. Impermeable seeds of alfalfa. Colo. Exp. Sta., Bull. 326. 1928.

265. Machacek, J. C. and Wallace, H. A. H. Non-sterile soil as a medium for tests of seed germination and seed-borne disease in cereals. Canad. Jour. Res., C, **20**: 539–557. 1942.

266. Mar, Frank. Amylase activity as an indication of seed viability. Unpub. Thesis, Ia. State Coll. Lib. 1944.

267. Mariat, F. Influence favorable de la Vitamine B sur les germinations de *Cattleya*. Rev. Hort. (Paris) **116**: 68–69. 1944.

268. Martin, A. C. The comparative internal morphology of seeds. Am. Mid. Nat. **36**: 513–660. 1946.

269. Martin, J. A. Jr. Germination of sweet potato seed as affected by different methods of scarification. Proc. Am. Soc. Hort. Sci. **47**: 387-390. 1946.

270. Martin, J. N. The strophiole in sweet clover and alfalfa seeds. Proc. Ia. Acad. Sci. **44**: 104. (1937) 1938.

271. ————. Germination studies of sweet clover seed. Ia. State Coll. Jour. Sci. **19**: 287–300. 1945.

272. ———— and Watt, J. R. The strophiole and other seed structures associated with hardness in *Melilotus alba* L. and *M. officinalis* Willd. Ia. State Coll. Jour. Sci. **18**: 457–469. 1944.

273. McAlister, Dean F. The effect of maturity on the viability and longevity of the seeds of western range and pasture grasses. Jour. Am. Soc. Agron. **35**: 442–453. 1943.

274. McGugan, Jean. Notes on the distinction of individuals of the genus *Brassica* by means of seeds and seedlings. Unpub. Thesis, Univ. Toronto. 1945.

275. Meehan, Frances. Species of *Helminthosporium* and *Gurvularia* associated with grass seeds. Unpub. Thesis, Ia. State Coll. Lib. 1947.

276. ————. A host index to seed-borne species of *Helminthosporium* and *Curvularia* on certain grasses. Assoc. Off. Seed Anal. **37**: 1947. [In press].

277. ———— and Murphy, H. C. A new *Helminthosporium* blight of oats.. Science 413-414. Nov. 1946.

278. Meginnis, H. G. Sulphuric acid treatment to increase germination of black locust seed. U.S. Dept. Agr. Circ. 454. 1937.

279. Meischke, Dieter. Über den Einfluss der Strahlung auf Licht und Dunkelkeimer. Jahrb. Wiss. Bot. **83**: 359–405. 1936.

280. Mercer, S. P. and Linehan, P. A. Experiments in the diagnosis of species and varieties of *Lolium* by the Gentner screened ultraviolet light method. Proc. Int. Seed Test. Assoc. **3** (18) : 180–191. 1931.

281. Meyer, J. R. The use of tomato juice in the preparation of a medium for the germination of orchid seeds. Am. Orchid Soc. Bull. **14**: 99–101. 1945. (1038).

282. Middleton, Gordon K. Hard seed in Korean *Lespedeza*. Jour. Am. Soc. Agron. **25**: 119–122. 1933.

283. ————. Size of Korean *Lespedeza* seed in relation to germination and hard seed. Jour. Am. Soc. Agron. **25**: 173–177. 1933.

284. Midgley, A. R. Effect of alternate freezing and thawing on impermeability of alfalfa and dodder seeds. Jour. Am. Soc. Agron. **18**: 1087–1098. 1928.

285. Miles, L. E. Effect of type and period of storage on cotton seed after treatment with organic mercury dusts. Phytopath. **29**: 986–991. 1939.

286. Mirov, N. T. A note on germination methods for coniferous species. Jour. For. **34**: 719–723. 1936.

287. Mohs, K. and Tornow, E. Germinating energy (of grains) and selenium reduction. Zeits. Ges. Getreidew. **27**: 17–22. 1940.

288. Moore, W. C. Seed Borne diseases. Am. Appl. Biol. **33**: 228–31. 1946.

289. Moss, E. H. Longevity of seed and establishment of seedlings in species of *Populus*. Bot. Gaz. **99**: 529–542. 1938.

290. Moutia, L. A. La teneur en humidité du mais en grain en relation avec divers états hygrométriques de l'air. Rev. Agr. Ile Maurice **21**: 122–126. 1942.

291. Muller, P. Beitrag zur Keimverbreitungsbiologie der Endozoochoren. Ber. Schweiz. Bot. Ges. 43: 241-252. 1943.

292. Munn, M. T. Further studies of the fungous associates of germination tests. Proc. Assoc. Off. Seed Anal. **13**: 57–59. 1921.

293. ————. Fluorescense readings of the strains of the species of *Lolium*. Proc. **29**: 136–137. 1937.

294. ————. Soil tests necessary to measure vitality of some seed stocks. Proc. **23–26**: 285–286. 1938.

295. ————. Germinating freshly harvested winter barley and wheat. Proc. **36**: 151–152.

296. Murley, Margaret. A seed key to 14 species of *Geraniaceae*. Proc. Ia. Acad. Sci. **51**: 1945.

297. ————. Distribution of Euphorbiaceae in Iowa, with seed keys. Ia. State Coll. Jour. Sci. **19**: 415–427. 1945.

298. ————. Fruit key to the Umbelliferae in Iowa, and plant distribution records. Ia. State Coll. Jour. Sci. **20**: 349–361. 1946.

299. Musil, Albina F. The classification of brassicas from seedling growth. U.S. Dept. Agr. Div. For. Crops & Dis. 1939.

300. ————. Weed seed identification. U.S. Dept. Agr. Bur. Pl. Ind. Div. Seed Inv. 1938.

301. ————. The pre-treatment of Buffalo grass for field planting. Proc. Assoc. Off. Seed Anal. **33**: 76–82. 1941.

302. ————. Diagnostic characters of seed of the commercial species of *Agrostis* and certain species occurring incidentally with them. Proc. Assoc. Off. Seed Anal. **34**: 139–151. (1943). 1942.

303. ————. Seeds of grasses cultivated for forage or occurring incidentally with crop seeds. Miscellaneous species. U.S. Dept. Agr. Bur. Pl. Ind., Soils and Agr. Eng. 1944.

304. ————. Seeds of grasses cultivated for forage or occurring incidentally with crop seeds. The genus *Setaria*. U.S. Dept. Agr., Bur. Pl. Ind., Soils and Agr. Eng. 1944.

305. ————. Seeds of dodder occurring with crop seeds. U.S. Dept. Agr., Div. Forage Crops & Dis. 1944.

306. ————. Seeds of grasses cultivated for forage or occurring incidentally with crop seeds. The genus *Panicum*. U.S. Dept. Agr., Bur. P. Ind. Soils & Agr. Eng. 1944.

307. ————. Distinguishing characters of the seeds of four species of *Agropyron*. U.S. Dept. Agr., Bur. P. Ind., Soils & Agr. Eng. 1946.

308. ————. The germination of guar, *Cyamopsis tetragonolobus* (L.) Taub. Jour. Am. Soc. Agron. **38**: 661–662. 1946.

309. ————. Distinguishing species of *Poa* from their seeds. U.S. Dept. Agr., Bur Pl. Ind., Soils & Agr. Eng. 1946.

310. ————. Distinguishing species of *Avena* from their seed. U.S. Dept. Agr., Bur. Pl. Ind., Soils & Agr. Eng. 1946.

311. Muskett, A. E. and Malone, J. P. The Ulster method for the examination of flax seed for the presence of seed borne parasites. Ann. Appl. Biol. **28**: 8–13. 1941.

312. Myers, Amy. Longevity of seed of natural grasses. Agr. Gaz. New So. Wales **51**: 405. 1940.

313. ————. Cold storage of onion seed. Agr. Gaz. New So. Wales 53: 528. 1942.

314. ————. Germination of curly mitchell grass *Astrebla lappacea* (Domin.). Jour. Australian Inst. Agr. Sci. 8: 31–32. 1942.

315. Natlacen, Guglielmo. I piu recenti progressi nella determinazione della germinbilita dei semi forestale. Alpe 25: 16–20. 1938.

316. Nazarova, N. S. Catalase activity as an index of the germinating capacity of seeds. Bull. Appl. Bot. Genet. & Pl. Breed., IV, 2: 111. 1937.

317. Neergaard, Paul. Seed-borne fungous diseases of horticultural plants. Proc. Int. Seed Test. Assoc. 12: 47–71. 1940.

318. ————. Danish species of *Alternaria* and *Stemphylium*. 560 pp.. 1945.

319. Neill, J. C. and Hyde, E. O. C. Blind-seed disease of ryegrass. New Zea. Jour. Sci. Tech. 20A: 281–301. 1939.

320. ————. Blind-seed disease of ryegrass. New Zea. Jour. Sci. Tech. 24A: 65–71. 1942.

321. Neljubow, D. Über die Methoden der Bestimmung der Keimfähigkeit ohne Keimprüfung. Ann. Essais Semences 4: 31–35. 1925.

322. Nelson, Mary L. Successful storage of southern pine seed for seven years. Jour. For. 38: 443–444. 1940.

323 Newton, J. M. and Naylor, N. M. Soybean amylase, I. The concentration and characterization of soybean amylase. Cereal Chem. 16: 71–78. 1939.

324. Niethammer, Anneliese. Kritisch. vergleichende. Zucker-und Katalasebestimmunfen an Samennaterial der verschiedensten Lebenskraft. Zeits. Pflanzenernährung Dugun. Bodenk. A. Wiss. Teil. 21: 69–86. 1931.

325. ————. Plamolysestudien an gärtnerisch wichegem Saatgut. Gartenbauviss 17: 91–94. 1942.

326. Nihous, M. Sur l'influence toxique der solutions aqueses de colchicine sur la germination des graines de *Pisus sativum,* (Pois cutlive). Compt. Rend. Soc. Biol. 138: 128. 1944.

327. Nilsson, Fredrik. Analyskvartslampans anvandning for atskiljande av. italienskt och engelski rajgras (*Lolium multiflorum* Lam. och. *L. perenne* L.) [Use of the analytical quartz Hg. vapor lamp for distinguishing Italian and per. rye grasses]. Severiges Utsadesforenings Tidskr. 43: 112–120. 1933.

328. ————. Eininge Resultate von Isolation und Bastardierungsversuchen mit *Lolium multiflorum* Lam. und *Lolium perenne* L. Bot. Not. 161–165. 1930.

329. Noble, Mary. Notes on *Pullularia pullulans* in ryegrass seed and seed testing methods as affecting detection of certain seed-borne diseases. Ann. Appl. Biol. 26: 630–633. 1939.

330. Norris, Elva L. Identification of sorghum. Proc. Assoc. Off. Seed Anal. 34: 151–155. (1943). 1942.

331. Nuckols, S. B. Plant Horome treatment of sugar beet seed. Sugar (New York) 37: 22–23. 1942.

332. Nutile, G. E. Inducing dormancy in lettuce seed with coumarin. Pl. Physiol. 20: 433–442. 1945.

333. ————. Growth and yield of baldhead bean plants in the field. Proc. Assoc. Off. Seed Anal. 36: 184–188. 1946.

334. Ogilvie, L., Croxall, H. E. and Hickman, C. J. Cuprous oxide as a seed protectant for peas. Ann. Rep. Agr. & Hort. Res. Sta. Univ. Bristol 1939: 88–99. 1940.

335. Olmo, H. P. Empty seededness in varieties of *Vitis vinifera*. Proc. Am. Soc. Hort. Sci. 32: 376–380. 1934. (1935).

336. ———. The use of seed characters in the identification of grape varieties. Proc. Am. Soc. Hort. Sci. **40**: 305–309. 1942.

337. Orton, C. R. Seed-borne parasites, a bibliography. W. Va. Agr. Exp. Sta., Bull. 245. 1931.

338. Ozorio de Almeida, A., Goulart, M. D., Ielpo, M. and Vierira Pinto, A. Estudo da acao inhebidora do suco de *Solanum lycopersicum* sobre a germinacao de sementes e crescimento de plantas. [The inhibiting action of the juice of tomato on seed germination and growth]. Rev. Brazil Biol. **1**: 345–354. 1941.

339. Penneff, Nicola. Die Überwindung der Hartschaligkeit beim Saatgut der *Robinia* (*Robinia pseudacacia* L.) Forstwiss. Centralbl. **63**: 232–237. 1941.

340. Pepin, A. and Wright, W. H. The identification of false wild oats in the seed form. Mimeographed. (No series identification) or State Dept.. Agr., Canada. [Mimeo].

341. Perez de Mattos, Horacio. Applicacao de hormonio sementes. Bol. Soc. Brasileira Agron. **5**: 65–77. 1942.

342. Petrova, D. A. Effect of alkali upon germinating capacity of seeds of Kok-saghyz and of common dandelion. Comp. Rend. (Dok.) Acad. Sci. USSR **49**: 227–228. 1943.

343. Pfeiffer, Norma E. Morphology of the seed of *Symphoricarpus racemosus* and the relation of fungal invasion of the seed coat to germination capacity. Contr. Boyce Thompson Inst. **6**: 103–122. 1934.

344. Phillis, E. and Mason, E. G. The effect of extreme desiccation on the viability of cotton seed. Ann. Bot. **9**: 353–359. 1945.

345. Piskareff, V. J. [Determining tree-fruits seed germinability by means of staining]. Michuriusko Plodovdstivo. [Res. Inst. Fruit Growing I. W. Mitchuren USSR.] **1937**: 61–62. 1937. [English summary].

346. Poptsov, A. O. Vtorichnyi pokoi u semian krymsagyza. Doklady Akademii Nauk USSR. [Compt. Rend. Acad. Sci. USSR.]. **2**: 593–597. 1935.

347. Porter, R. H. Dry rot of corn and its control. Ia. Agr. Exp. Sta., Serv. Bull. 114. 1923.

348. ———. Reaction of cucumbers to types of mosaic. Ia. State Coll. Jour. Sci. **6**: 95–129. 1931.

349. ———. Pathological aspects of seed testing. Proc. Assoc. Off. Seed Anal. **23/26**: 128–131. 1930–33. (1938).

350. ———. Detection of seed-borne parasites. Proc. Assoc. Off. Seed Anal. **23/26**: 218–222. 1930–33. (1938).

351. ———. Effect of seed-borne pathogens and of seed disinfectants on the germination of barley seed. Proc. Assoc. Off. Seed Anal. **27**: 94–99. 1935.

352. ———. Germination of black locust seed. Proc. Assoc. Off. Seed Anal. **27**: 63–65. 1935.

353. ———. Relation of seed disinfectants to seed analysis. Proc. Assoc. Off. Seed Anal. **28**: 93–201. 1936.

354. ———. Application of the Chi-square test to purity analysis of blue grass seed. Proc. Assoc. Off. Seed Anal. **29**: 91–93. 1937.

355. ———. Germination of domestic ryegrass seed. Assoc. Off. Seed Anal. News Letter **11**: 5–6. 1937.

356. ———. Uniform techniques for the analysis of small seeded grasses. Proc. Assoc. Off. Seed Anal. **30**: 133–171. 1938.

357. ———. Detection and classification of seed-borne organisms, their effect on germination and their control by seed disinfection in laboratory and field. Proc. Assoc. Off. Seed Anal. **30**: 195–213. 1938.

358. ————. Experiments with modified techniques for the determination of purity and viability of blue grass seed, *Poa pratensis* L. Ia. Agr. Exp. Sta., Res. Bull. 235. 1938.

359. ————. Summary of tests with orchard grass using the Iowa method as studied by Norma K. Seiferle in the Iowa State College Seed Laboratory. Assoc. Off. Seed Anal., News Letter **14**: 2–11. 1940.

360. ————. Our responsibilities as an Association. Presidential address, Assoc. Off. Seed Anal., Ann. Meet. 1940.

361. ————. Seed-borne organisms and plant quarantines. Jour. Econ. Ent. **34**: 543–548. 1941.

362. ————. Testing the quality of seeds for farm and garden. Ia. Agr. Exp. Sta., Res. Bull. 334. 1944.

363. ————. Induced baldhead in soybeans. Phytopath. **36**: 168–170. 1946.

364. ————. Germinability of treated and untreated lots of vegetable crop seeds in *Pythium*-infested soil and in the field. Ia. Agr. Exp. Sta., Res. Bull. 345. 1946.

365. ———— and Durrell, Mary and Romm, H. J. The use of 2, 3, 5-triphenyl-tetrazolium-chloride as a measure of seed germinability. Pl. Physiol. **22**: 149–159. 1947.

366. ———— and Hendershott, W. E. and Davis, G. N. Indexing farmers' seed lots for seed-borne organisms and response to seed disinfectants. Ia. Agr. Exp. Sta., Res. Bull. 238. 1938.

367. ———— and Koos, Katherine. Germination of injured weed and crop seed. Proc. Assoc. Off. Seed Anal. **28**: 68–73. 1936.

368. ———— and Leggatt, C. W. A new concept of pure seed as applied to seed technology. Sci. Agr. **23**: 80–103. 1942.

369. Preston, R. D. and Scott, L. J. The significance of the micropyle in relation to water entry in some leguminous seeds. Proc. Leeds Phil. & Lit. Soc. **4**: 123–134. 1943.

370. Prince, A. E. Fungi isolated from peanuts collected from South Carolina in 1944. Pl. Dis. Rep. **29**: 367–368. 1945.

371. Raleigh, G. J. Chemical conditions in maturation, dormancy and germination of seeds of *Gymnocladus dioica*. Bot. Gaz. **89**: 284–300. 1943.

372. Rampton, H. H. The use of the fluorescence test with ultra-violet light in classifying the ryegrasses (*Lolium* spp.) of western Oregon. Jour. Am. Soc. Agron. **30**: 915–922. 1938.

373. Randolf, L. F. and Cox, Leland G. Factors influencing the germination of *Iris* seed and the relation of inhibiting substances to embryo dormancy. Proc. Am. Soc. Hort. Sci. **43**: 284–300. 1943.

374. Ransom, Elizabeth Ruth. The inter-relations of catalase, respiration, after-ripening and germination in some dormant seeds of the *Polygonaceae*. Am. Jour. Bot. **22**: 815–825. 1935.

375. Ray, W. W. and McLaughlin, J. H. Isolation and infection tests with seed- and soil-borne cotton pathogens. Phytopath. **32**: 233. 1942.

376. Reddy, C. S. and Rice, W. N. Seed treatment of good seed corn. Phytopath. **32**: 23. 1942.

377. Reinhard, A. W. Zur Frage der Samenkeimung bei *Solanum lycopersicum*. Zeitschd. Wiss. Biol., E, **20**: 792–796. 1933.

378. Reports of Iowa Agr. Exp. Sta. Proj. 427. 1941: 34–35. 1943: 31–32. 1944: 38–39. 1946: 33.

379. Reports of Research Committee. Proc. Assoc. Off. Seed Anal. 1935: 16. 1936: 16. 1937: 52–53. 1938: 21–24; 30 and 38; 40–46; 52. 1939: 43–45, 46–47, 48–51.

380. Reports of Special Committee on Standardized tests. Proc. Assoc. Off. Seed Anal. 1942: 25–26. 1943–1944: 69–74.

381. Reports of Sub-committee on Analysis of small seeded grasses. Proc. Assoc. Off. Seed Anal. 1942: 32–33. 1943: 56–57.

382. Reports of sub-committee on brome grass analysis. Proc. Assoc. Off. Seed Anal. 1938: 47–49. 1939: 66–67. 1940: 30–31. 1941: 24–25.

383. Reports of sub-committee on sand tests. Proc. Assoc. Off. Seed Anal. 1941: 33–34. 1942: 42–46.

384. Reports of sub-committee on viability of weed seeds. Proc. Assoc. Off. Seed. Anal. 1938: 52–57. 1939: 68–72. 1940: 31–42. 1941: 33–34. 1942: 44–46. 1943: 67–68.

385. Retovsky, R. Verkürzung der Ruheperiode der Samen von *Aesculus hippocastanum* L. durch Uranylnitrat. Ber. Deut. Bot. Ges. **6**: 355–366. 1942.

386. Rice, W. N. The haemocytometer method for detecting fungous spore load carried by wheat seeds. Proc. Assoc. Off. Seed Anal. **31**: 124–127. 1939.

387. ———. The development and evaluation of seed laboratory methods for determining the presence and significance of certain soil-borne and seed-borne organisms. Unpub. Thesis, Ia. State Coll. Lib. 1944.

388. Rivera, Rafael, Popp, H. W. and Dow, R. B. The effect of hydrostatic pressures upon seed germination. Am. Jour. Bot. **24**: 508–513. 1937.

389. Robbins, Wayne A. The Iowa experimental method for purity analysis of chalcid-fly infested red clover and alfalfa seeds. Proc. Assoc. Off. Seed Anal. **32**: 46–49. 1940.

390. ——— and Porter, R. H. Germinability of sorghum and soybean seed exposed to low temperatures. Jour. Am. Soc. Agron. **38**: 905–913. 1946.

391. Roberts, R. Alan and Thomas, J. O. A study of the distinguishing features of the seeds and seedlings of some farm *Cruciferae*. Proc. Int. Seed Test. Assn. **5**: 113–131. 1933.

392. Robertson, D. W., Lute, Anna M. and Kroeger H. Germination of 20-year old wheat, oats, barley, rye, sorghum, and soybeans. Jour. Am. Soc. Agron. **35**: 786–795. 1943.

393. Robson, W. S. The seedling characters of some cultivated *Brassicae*. Ann. Appl. Biol. **21**: 418–429. 1934.

394. Roe, Eugene I. Longevity of red pine seed. Proc. Minn. Acad. Sci. **8**: 28–30. 1940.

395. Rogers, M. Susana. Seed germination experiments with fragrant dwarf indigo, *Amorpha nana*. Colo. Univ. Stud. **18**: 205–213. 1930–31.

396. Rohmeder, Ernst. Das Selvenverfahren als Hilfsmittel der forstlichen Saatgutbeurteilung. Deut. Forstwirt. **22**: 33–35. 1940.

397. ———. Keimversuche mit *Ulmus montana* (With.). Forstwiss. Centralbl. **64**: 122–135. 1942.

398. Rose, R. E. Germination and conidial number relationship in blind-seed disease. New Zeal. Jour. Sci. Tech., A, **27**: 255–257. 1945.

399. Rules and regulations relating to the Iowa Seed Law. 1941. State Department of Agriculture, Des Moines, Iowa.

400. Rules and regulations under the Federal Seed Act. 1939.

401. Russell, R. C. and Ledingham, R. J. Wheat seed testing from the pathological standpoint with special reference to embryo exposure. Sci. Agr. **21**: 761–774. 1941.

402. Sagaguchi, Isamu. Relation between maturation of fruit flesh and development of seeds in peach. Proc. Crop Sci. Soc. Japan **2**: 96–103. 1930.

403. Sass, John E. Histological and cylotogical studies of mercury phosphate poisoning in corn seedlings. Phytopath. **27**: 95–99. 1937.

404. Sayre, J. D. Storage tests with seed corn. Ohio Jour. Sci. **40**: 181–185. 1940.

405. Schaefer-Safonova, E. J., Kalaschimkova, M. I. and Kostromina, A. S. Determination of the viability of tree seeds by means of the staining method. Jour. Bot. USSR. **19**: 593–594. 1934.

406. Schjelderup-Ebbe, Thorlief. Über die Lebensfähigkeit alter Samen. Skrifter Norske Vidensk. Akad., Oslo Mat. Nalurvidensk Kl. **1935**: 1–178. 1936.

407. Schroeder, Eltora M. Dormancy in seeds of *Benzion aestivale* L. Contr. Boyce Thompson Inst. **7**: 411–419. 1935.

408. Schwemmle, J. [Germination tests with old seeds]. Zeits. Bot. **36**: 225–261. 1940.

409. Schwendiman, Alvin, and Shands, H. L. Delayed germination or seed dormancy in Vicland oats. Jour. Am. Soc. Agron. **35**: 681–688. 1943.

410. Scott, D. H., Waugh, J. G. and Cullinan, F. P. An injurious effect of peach juice on germination of the seed. Proc. Am. Soc. Hort. Sci. **40**: 283–285. 1942.

411. Seiferle, Norma K. and Porter, R. H. A possible modification in purity analyses of orchard grass. Proc. Assoc. Off. Seed Anal. **29**: 94–96. 1937.

412. Sell, O. E. Scarified bur becomes miracle clover. Southeastern Seedsman **7**: 12, 61, 67. 1944.

413. Shands, R. G. Longevity of *Gibberella saubinetii* and other fungi in barley kernels and its relation to the emetic effect. Phyopath. **27**: 749–762. 1937.

414. Sharpe, Ralph H. and Merrill, Samuel Jr. Effect of stratification and time of planting on germination of tung seed. Proc. Am. Soc. Hort. Sci. **40**: 286–291. 1942.

415. Shoemaker, D. N. and Delwicke, E. J. Description of types of principal American pea varieties. U. S. Dept. Agr. Misc. Publ. 170. 1934.

416. Shuck, A. L. Some factors influencing the germination of lettuce seed in seed laboratory practice. N. Y. (Geneva) Agr. Exp. Sta., Bull. 222. 1934.

417. ————. A growth-inhibiting substance in lettuce seeds. Science **81**: 236. 1935.

418. ————. Light as a factor influencing the dormancy of lettuce seeds. Pl. Physiol. **10**: 193–196. 1935.

419. Silow, R. A. A systemic disease of red clover caused by *Botrytis anthophila* Bond. Trans. Brit. Myc. Soc. **18**: 239–248. 1933.

420. Simmonds, P. M. Detection of loose smut fungi in embryos of barley and wheat. Sci. Agr. **26**: 51–58. 1946.

421. Simpson, D. M. Dormancy and maturity of cotton seed. Jour. Agr. Res. **50**: 429–434. 1935.

422. ————. Factors affecting the longevity of cotton seed. Jour. Agr. Res. **64**: 407–419. 1942.

423. ————. The longevity of cotton seed as affected by climate and seed treatments. Jour. Am. Soc. Agron. **38**: 32–45. 1946.

424. ————, Adams, Caroline, L. and Stone, G. M. Anatomical structure of the cotton seed coat as related to problems of germination. U. S. Dept. Agr., Tech. Bull. 734. 1940.

425. Sing, B. N., Mathur, P. B. and Merta, M. L. Determination of catalase ratio as a rapid method of seed testing. Trop. Agr. (Trinidad) **15**: 260–81. 1938.

426. Snedecor, G. W. Statistical methods. 4ed. 1946.
427. Snyder, W. C. Seed dissemination in *Fusarium* wilt of pea. Phytopath 22: 253–257. 1932.
428. ————. A leaf, stem, and pod spot of pea caused by a species of *Cladosporium*. Phytopath. 24: 890–905. 1934.
429. Spaeth, J. Nelson. A Physiological study of dormancy in *Tilia* seed. N. Y. (Cornell) Agr. Exp. Sta., Mem. 169. 1934.
430. Spanier, F. H., Porter, R. H. and Durell, Mary. What constitutes pure seed in tobacco seed? Assoc. Off. Seed Anal., News Letter 20 (2): 24–27. 1946.
431. Sprague, G. E. The relation of moisture content and time of harvest to germination of immature corn. Jour. Am. Soc. Agron. 28: 472–478. 1936.
432. Sprague, V. G. Germination of freshly harvested seeds of several *Poa* species and of *Dactylis glomerata*. Jour. Am. Soc. Agron. 32: 715–721. 1940.
433. Stahl, Chr. Comparative experiments between the laboratory and the field germination of seed. Int. Seed Test. Assoc. Proc. 15–17: 75–143. 1931.
434. Stahler, L. M. Preliminary work on germination of *Agropyron* caryopses. Proc. Assoc. Off. Seed Anal. 27: 56–59. 1936.
435. Steinbauer, C. E. Methods of scarifying sweet potato seed. Proc. Am. Soc. Hort. Sci. 35: 606–608. 1937 (1938).
436. Steinbauer, George P. Dormancy and germination of *Fraxinus* seeds. Pl. Physiol. 12: 813–824. 1937.
437. Steinmetz, F. H. and Arny, A. C. A classification of the varieties of field beans. Jour. Agr. Res. 45: 1–50. 1932.
438. Stevens, O. A. and Long, H. D. Sweet clover seed studies. No. Dak. Agr. Exp. Sta., Bull. 197. 1926.
439. Stewart, William S. and Hamner, Charles L. Treatment of seeds with synthetic growth-regulating substances. Bot Gaz. 104: 338–347.
440. Stoddart, L. A. and Wilkinson, K. J. Inducing germination in *Oryzopsis hymenoides* for range re-seeding. Jour. Am. Soc. Agron. 30: 763–768. 1938.
441. Stout, Myron and Solman, Bion. Interference of ammonia released from sugar beet seed balls with laboratory germination tests. Jour. Am. Soc. Agron. 33: 65–69. 1941.
442. Stuart, Neil W. Expanded vermiculite for seed germination and plant growth. Florists Exchange 105: 18. 1945.
443. Swarthley, John C. and Chadwick, L. C. Effects of synthetic growth substances on cuttings, seeds and transplants. Ohio Agr. Exp. Sta., Bimo. Bull. 27: 125–144. 1942.
444. Takagi, Ichizo. On the storage of mulberry seeds. Res. Bull. Tokyo Imp. Ser. Coll. 2: 1–22. 1939.
445. Tatum, L. A. and Zuber, M. S. Germination of maize under adverse conditions. Jour. Agr. Res. 35: 48–59. 1943.
446. Taubenhaus, J. J. Seeds of watermelon and okra as possible carriers of *Fusarium* wilt. Phytopath. 25: 969. 1935. [abstract].
447. Taylor, Carlton F. and Rupert, Joseph A. A study of vegetable seed protectants. Phytopath. 36: 726–749. 1946.
448. Tervet, I. W. The influence of fungi on storage, on seed viability and on seedling vigor of soybeans. Phytopath. 35: 3–15. 1945.
449. Thomas, H. R. Cercospora blight of carrot. Phytopath. 33: 114–125. 1943.
450. Thompson, Ross C. Some factors associated with dormancy of lettuce seed. Proc. Am. Soc. Hort. Sci. 33: 610–616. 1935.

451. ————. Germination of endive seed (*Cichorium endiva*) at high temperatures stimulated by thiourea and by water treatments. Proc. Am. Soc. Hort. Sci. **47**: 323–326. 1946.

452. ———— and Horn, Norman L. Germination of lettuce seed at high temperatures (25–35° C) stimulated by thiourea. Proc. Am. Soc. Hort. Sci. **43**: 431–439. 1944.

453. ———— and Kosar, William F. Stimulation of germination of dormant lettuce seed by sulphur compounds. Pl. Physiol. **14**: 567–573. 1939.

454. Thornton, Norwood C. Factors influencing germination and development of dormancy in cocklebur seeds. Contr. Boyce Thompson Inst. **7**: 477–496. 1935.

455. ————. Carbon dioxide storage. IX. Germination of lettuce seeds at high temperatures in both light and darkness. Contr. Boyce Thompson Inst. **8**: 25–40. 1936

456. ————. Carbon dioxide storage. XII. Germination of seeds in the presence of carbon dioxide. Contr. Boyce Thompson Inst. **13**: 355–360. 1944.

457. ————. Importance of oxygen supply in secondary dormancy and its relation to the inhibiting mechanism regulating dormancy. Contr. Boyce Thompson Inst. **13**: 487–500. 1945.

458. Toole, Eben H. Observations on the germination of freshly harvested timothy seed. Proc. Int. Seed. Test. Assoc. **11**: 119–139. 1939.

459. ———— and Brown, E. Final results of the Duvel buried seed experiment. Jour. Agr. Res. **72**: 201–210. 1946.

460. ———— and Coffman, F. A. Variations in the dormancy of seeds of the wild oat, *Avena fatua*. Jour. Am. Soc. Agron. **32**: 631–638. 1940.

461. Toole, Vivian K. The germination of seed of *Oryzopsis hymenoides*. Jour. Am. Soc. Agron. **32**: 33–41. 1940.

462. ————. Germination of seed of vine-mesquite, *Panicum obtusum*, and plains bristle-grass, *Setaria macrostachya*. Jour. Am. Soc. Agron. **32**: 503–512. 1940.

463. ————. Factors affecting the germination of various dropseed grasses. (*Sporobolus* spp.) Jour. Agr. Res. **62**: 691–715. 1941.

464. Trumble, H. C. and Phipps, I. B. The inheritance of fluorescence in hybrids between perennial and Wimmera ryegrass. Jour. Australia Counc. Sci. & Ind. Res. **6**: 170–178. 1933.

465. Tskhoidze, V. Opredelenie oskhozhesti semian tungovogo dereva metodom okrashivaniia [Determining the viability of tung-oil seeds by straining]. [In Russ. with Eng. summ.] Izvestiia Batumskog Subtropicheskogo Botanicheskogo Sada **1**: 91–96. 1936.

466. Tukey, H. B. and Barret, M. S. Approximate germination test for non-after-ripened peach seed. Pl. Physiol. **11**: 629–633. 1936.

467. ———— and Carlson, R. F. Breaking the dormancy of peach seed by treatment with thiourea. Pl. Physiol. **20**: 505–516. 1945.

468. Tullis, E. C. Fungi isolated from discolored rice kernels. U. S. Dept. Agr., Tech. Bull. 540. 1936.

469. Turner, J. H. The viability of seeds. Kew. Bull. Misc. Inf. **6**: 257–269. 1933.

470. Ultee, A. J. Het bewaren van zaadkoffie. Arch. Koffie Cultuur Ned.-Indie **7**: 75–83. 1933.

471. Valleau, W. D. Two seed-transmitted ring-spot diseases of tobacco. Phytopath. **22**: 28. 1932. [Abstract].

472. ————. Symptoms of yellow ring-spot and longevity of the virus in tobacco seed. Phytopath. **29**: 549–551. 1939.

473. Vinall, H. M., Stevens, J. C. and Martin, J. H. Identification, history and distribution of common sorghum varieties. U. S. Dept. Agr., Tech. Bull. 506. 1936.

474. Vincent, G. Die Aufbewahrung der Fichten und Lärchensamen. Zeit. Forst. u. Jagdwesen **70**: 45–51. 1938.

475. Waal, D. Het ondersheiden van der Nedelansche tarwerassen aan het zaad door de kluerreactie met phenol. Landbouwk. Tijds. **52**: 753–760. 1940.

476. Wach, Alfred. Versuche zur Selenitfärbung des forstlichen Saatgutes. Allg. Forst. u. Jagdstg. **118**: 178–188. 1942.

477. Wade, B. L. A key to pea varieties. U. S. Dept. Agr., Circ. 676. 1943.

478. Watt, John R. Development and impermeability of seeds of sweet clover. Unpub. Thesis, Ia. State Coll. Lib.

479. Watts, Victor M. Rest period in cucumber seeds. Proc. Am. Soc. Hort. Sci. **36**: 652–654. 1938.

480. Webster, C. B. and Ratliffe, Geo. T. A method of forcing quick germination of *Juniperus virginiana* L. seed. Jour. For. **40**: 268. 1942.

481. Weetman, Lesie M. Inheritance and correlation of shape, size and color in the watermelon, *Citrullus vulgaris* Schrad. Ia. Agri. Exp. Sta., Res. Bull. 228. 1937.

482. Weihing, Ralph M. Field germination of alfalfa seed submitted for registration in Colorado and varying in hard seed content. Jour. Am. Soc. Agron. **32**: 944–949. 1940.

483. Weise, Rudolf. Bermerkungen zu Gurewitsch's Methode, die Keimfähigkeit von Samen zu bestimmen. Ber. Deut. Bot. Ges. **55**: 338–340. 1937.

484. Wenger, Leon. Soaking Buffalo grass (*Buchloë datyloides*) seed to improve its germination. Jour. Am. Soc. Agron. **33**: 135–141. 1931.

485. Wester, R. E. Comparison of yield between normal and baldhead snap bean plants. Proc. Assoc. Off. Seed Anal. **36**: 172–177. 1946.

486. Whitcomb, W. O. Hard seeds in legumes. Mont. Agr. Exp. Sta., Bull. 248. 1941.

487. White, N. H. and Dickson, B. T. A spot disease of guayule (*Parthenium argentatum* Gray). Jour. Australia Counc. Sci. & Ind. Res. **16**: 258–260. 1943.

488. Wiesehuegel, E. G. Germinating Kentucky coffee tree. Jour. For. **33**: 533–534. 1935.

489. Wilson, Coyt. Damaged peanuts in Alabama in 1945. Pl. Dis. Rep. **30**: 12–13. 1946.

490. Wilson, J. J. The pathological relationship between the host and parasite in varieties and strains of watermelons resistant to *Fusarium niveum* E. F. S. Ia. Agr. Exp. Sta., Res. Bull. 195. 1936.

491 Wilson, J. K. Immersing seeds of species of *Robinia* in boiling water hastens germination. Jour. For. **42**: 453–454. 1944.

492. Wilson, M., Noble, Mary and Gray, Elizabeth. Blind-seed disease of ryegrass. Nature **145**: 783. 1940.

493. ————, ———— and ————. The blind-seed disease of ryegrass and its causal fungus. Trans. Royal Soc. Edinb. 51 Part 2 (No. 12): 327–340. 1945.

494. Witte, Hernfrid. Some international investigations regarding hard leguminous seeds and their value. Proc. Int. Seed Test. Assoc. **6**: 279–312. 1934.

495. Woodbridge, Mary E. A study of the rate of occurrence of certain weed seeds in replicate analyses of the seed of timothy. (*Phleum pratense*) Proc. Int. Seed Test. Assoc. **11**: 5–24. 1939.

496. Woodforde, A. H. The inheritance of a substance in the roots of seedling hybrid derivatives of *Lolium perenne* L. and *Lolium multiflorum* Lam. Jour. Linn. Soc. So. London **50**: 141–151. 1935.

497. Wright, W. H. Hulled seeds of *Ambrosia artemisiifolia* L., and *Agropyron repens* L. Beauv. Assoc. Off. Seed Anal. News Letter **10**: 5–6. 1936.

498. ————. The quicker and the stronger methods. Proc. Int. Seed Test. Assoc. **10**: 299–301. 1938.

499. Youden, W. J. Seed treatment with talc and root-inducing substances. Contr. Boyce Thompson Inst. **11**: 207–218. 1940.

500. Youngman, Wilbur H. America—home of the bean. Agr. in the Americas **3**: 228–232. 1943.

501. Zahnley, J. W. Research seed projects. Kan. State Bd. Agr., 28th Bienn. Rep., **33**: 191–194. 1931–1932. (1933).

502. Zybina, S. P. Opytnaia rabota po izucheniiu balezni 18 na v Nizhegorodskoi gub. v. 1927–28. Bolezni Rastenii (Morbi Plantarum) **18** (½): 67–100. 1929.

THE BOTANICAL REVIEW

VOL. XV JUNE, 1949 No. 6

THE CHEMOTHERAPY OF PLANT DISEASES[1]

E. M. STODDARD AND A. E. DIMOND
Connecticut Agricultural Experiment Station

INTRODUCTION

The concept of chemotherapy as applied to control of plant diseases is centuries old, and one can merely guess as to its origin. Actual control of plant diseases, however, even to a slight extent, is a modern achievement, and this paper is intended to discuss some of the concepts and experimental progress which have been made toward that end.

Great impetus to studies of chemotherapy of plant diseases has resulted from the successful use of antibiotic and sulfa drugs for man and other warm-blooded animals, but progress has been slower in plant than in animal investigations for two primary reasons. Plants lack the forced circulatory system which in animals results in rapid and reasonably uniform distribution of a chemotherapeutic agent. Plants also lack the generalized mechanisms for combating infection and establishing immunity which are present in animals. The fact that most plants are of low economic value as individuals is important only because it has discouraged investigations which must commence with attention to the individual plant. Despite these barriers to rapid exploration, chemotherapy of plant diseases is a fertile field for research.

The word "chemotherapy" means simply the cure of existing disease by means of chemicals. Usage has narrowed the term to mean control of disease by chemicals which are introduced into the plant. In this paper the concept includes control of disease by chemicals, however applied, so long as the chemical finds its way into and exerts its ultimate action inside the host plant. Thus foliage sprays, which do not penetrate appreciably beneath the surface of the leaf, are excluded from this definition, but eradicative fungicides illustrate a type of chemotherapy.

---

[1] Published with the approval of the Director of the Connecticut Agricultural Experiment Station.

Perhaps a distinction should be made between therapy of a sick plant and prevention of disease in a healthy plant by introduced chemicals. At present this distinction has little practical meaning in relation to chemotherapy, since if treatment with chemical and exposure to disease are at all close together in point of time, a distinction between therapy and prophylaxis is difficult if not impossible to make. Treatment of a plant with chemical is not effectively accomplished the instant that the chemical is applied, but only after it has become distributed. The plant may be inoculated at a particular instant, but the time when it becomes diseased cannot be stated. This being true, no attempt is made here to distinguish prophylactic from chemotherapeutic action.

The scope of the present paper is somewhat narrower than is implied by the above definition. Plants suffering from deficiency diseases can certainly be treated and cured through chemotherapy, but such diseases of physiologic origin are not discussed in the present paper, their symptoms, experimental diagnosis and control having been reviewed elsewhere (35, 64). Nor is the use of chemotherapeutic techniques in combating insect and related pests included, but instead the use of these methods against plant parasites of virus, bacterial and fungus origin.

The most promising types of plant disease against which chemotherapeutic attack may be directed are those which are essentially internal and systemic, and for which there is presently no satisfactory control. Vascular wilt diseases of fungus and bacterial origin and virus diseases generally are examples. A recent paper (55) has emphasized the importance of such types of disease and remarked upon the fact that they are now controlled only through the use of resistant varieties and crop rotation.

HISTORY

No attempt is made here to do more than survey the historical highlights of this large subject. Detailed accounts of special aspects of the topic are to be found elsewhere (54, 60, 64).

Just when the idea of internal treatment of plants was first seriously considered is probably not known. Certainly it is very old. Ibn-al-Awam records that during the 12th century attempts were made to color flowers and to flavor and perfume fruits either by inserting dry powder into the pith of the split root or stem, or by placing it under the bark. Leonardo da Vinci, during the 15th century and probably in ignorance of the earlier work, explored the possibility of introducing arsenical and other poisonous solutions into the trunks of trees apparently for the purpose of poisoning the fruit. This was the earliest recorded attempt to inject solutions of chemicals into tree trunks through bore holes.

Until the 17th century chemicals were put into plants for immediately practical reasons and without concern for how the plant functions. Perhaps the first reference to work on injection of plants with the welfare of the subject in mind is that cited by Roach's excellent review (64) as an anonymous contribution in 1602, followed by a second citation in 1765 to combating insects attacking trees or shrubs by introducing a powder of pepper, laurel or incense, in the first case, or metallic mercury, in the second, into a hole in the tree. Both of these, it is to be noted, involved the injection of insoluble materials, and whatever the motivation of the authors, it is questionable that either method had more than psychological value for the man applying it to his plants.

Historically and logically, the next trend in research on placing materials in plants arose from studies of the ascent of sap, the transpiration stream and root pressure. Magnol (cited by Roach (64)) explored the pathway of water up the stem by introducing dyes into the transpiration stream. Hales (34) made a study of the pressure differential between the atmosphere and the water columns within the stem, and emphasized the conditions resulting in root pressure and ascent of sap. This work was followed during the 19th century by a number of investigations using dyes and inorganic salts for the purpose of tracing the course of water movement (32, 57, 70).

It is of interest to note the work of Goff (31) who, at the end of the 19th century, severely root pruned young trees and attached rubber tubing to their cut roots which he connected to a flask filled with water and held above the top of the tree. The hydrostatic head of water caused swelling and expansion of buds up to the top of the tree in a very short time, whereas transplanted trees with only natural water supply lagged by many weeks. As a means of distributing chemicals in plants under specialized conditions, the experiment is pertinent here.

So intimately linked with the foregoing trend in research as to be indistinguishable from it is that resulting from the influence of Liebig and the agricultural chemists. Application of inorganic fertilizers to plants and the search for the ideal fertilizer emphasized inorganic nutrition of plants and brought with it a growing awareness of deficiency diseases. As relating to the present paper, the trend began with Hartig (37) in 1853, who injected trunks of trees through bore holes from a container outside the tree, and who, with growing interest to botanists of the time, continued this work. Sachs (71) appears to have been the first to have used the method to correct a deficiency disease by injecting iron salts in solution into a deficient tree, but he noted that distribution of the salts was poor, as indicated by the response of the tree.

The same method has since been used to introduce either complete or single salt nutrient solutions, down to the present time, and has culminated in the work of Roach (64–66) who has perfected the method for rapidly diagnosing and correcting deficiency diseases of plants.

Historically, pest control is the most recent application of internal treatment of plants. While it antedated plant nutrition, early studies in this direction were wholly empirical and involved introduction of insoluble solids into the pith and heartwood. The more scientific approach to pest control through internal treatment of plants appears to have begun with Shevyrev (72). He contributed more by stimulating other workers than through his own contributions to pest control as such. His own studies dealt largely with methods of introducing chemicals into trees and relating distribution of chemical to method of injection.

Under the influence of Shevyrev, Mokrzecki (58, 59) injected various nutrient solutions and observed that the intensity of attack by scale insects on apple and pear appeared to be related to treatment. In an effort to control attack by insect pests, considerable effort was made by various investigators to find the value of KCN solutions injected into the tree. Perosino (cited by Roach (64)) noted reduction in amount of scale insects on *Euonymus* and of phylloxera on grape, but effectiveness of the technique was related to time of year that treatment was made and to method of injection.

The dark ages of chemotherapy, at least in the United States, occurred during the years of the first World War, when, according to Surface (88) and Flint (26), commercial tree experts placed pills of KCN, $KClO_3$ and $FeSO_4$ into the trunks of trees, claiming miraculous cures of all sorts. The treatment was wholly worthless.

Only within the last decade have scientists again approached the technique with an open mind, and it is becoming clearly evident that condemnation of the foregoing method has been wholly unjustified. Muller (60) made extensive experiments in the field, directed at control of both insect pests and plant parasites. His work and the results of numerous others make one wonder very much why so many results of great promise were published through the last fifty years, only to be abandoned without further investigation.

A number of distinct problems are involved in chemotherapeutic investigation. Development of screening procedures, choice of method of introducing the chemical into the plant, study of distribution of chemicals once they are introduced, and finally, the results which one may expect of the technique are aspects of the problem discussed in the present paper.

MODES OF CHEMOTHERAPEUTIC ACTION

Plant pathologists now stand, in relation to chemotherapy, where they were in 1930 in relation to protective fungicides. At that time inorganic copper and sulfur fungicides constituted the materials used as protective sprays. There were no rapid screening procedures for finding new fungicides, and very little was known about the relation between chemical structure and fungicidal activity. Yet the use of protective fungicides to control plant diseases was an old firmly grounded procedure. Choice of a screening procedure for protective fungicides is relatively easy; one wishes to kill the fungus spore, or inactivate it, before it enters the leaf. But the procedure for selecting chemotherapeutic materials is by no means so obvious, and the design of test precedures depends upon how the chemical produces its effect in the plant. It is for this reason that it seems desirable to speculate upon possible modes of chemotherapeutic action. Some generalized mechanisms are described below by which chemicals might act against viruses, on the one hand, and against fungi and bacteria, on the other.

Virus Diseases

a. Inactivating Virus in vivo. For many years plant pathologists have appreciated that viruses can be inactivated *in vitro,* and have looked forward to the time when such inactivation could be accomplished in the plant without injury to the host. Experiments with X-disease of peach have already indicated that this can be done when dormant diseased buds are treated with heat or certain chemicals and then budded into healthy seedlings (79). Inactivation could be either irreversible or reversible, these two types of inactivation being comparable to fungicidal (permanent inactivation) and fungistatic activity (temporary inactivation).

b. Preventing Virus Multiplication. A chemotherapeutant might act by causing the virus to cease multiplying. Such activity could be either permanent (the virus could irreversibly lose its ability to multiply and the host would then show symptoms only in the area infected when treatment began) or temporary (the virus could regain its ability to multiply within the host as soon as the chemotherapeutant was diluted below the minimal inactivating titer). Thus Takahashi (89*a*), using the detached leaf tissue technique and assaying the virus concentration on *Nicotiana glutinosa,* showed that malachite green will act in this way against tobacco mosaic virus. That the host is involved in the inactivation of virus is indicated by the fact that malachite green has no effect on the virus *in vitro.*

c. Causing the Host to Become Resistant or Immune to Virus Disease. In such a case the chemical may participate in interaction with the living host so that it resists infection or can recover from a contracted virus disease. The experiments on X-disease of peach in which sulfanilamide, zinc sulfate and hydroquinone were used as chemotherapeutic agents are possible examples of this type of activity (83).

Diseases of Bacterial and Fungus Origin

The means by which chemicals may act internally in plants against parasitic diseases are more numerous than for virus diseases but no less easy to distinguish.

a. Inactivating the Parasite. As with fungicidal sprays, a compound may kill or inhibit the causal organism within the host. Needless to say, the requirements for a compound to act in this way differ considerably from those for a foliage spray. The material must be less phytotoxic, more soluble and more toxic to the parasite than is required for foliage sprays. It is probable that the effect of 8-quinolinol benzoate against Dutch elm disease (105) is an example of such activity.

b. Preventing Reproduction of the Parasite. Many diseases become economic problems, not because they occur in a few plants, but because they may reach epiphytotic proportions in a short period of time if weather and other conditions favor development of disease. In such a case the fungus must reproduce rapidly, and usually does so by producing spores. If sporulation could be prevented, an epiphytotic would not develop. Steinberg (78) has accumulated evidence that certain chemicals, particularly of the anthracene series, inhibit the fruiting of *Aspergillus niger,* and that traces of elements may become necessary for proper formation of spores (75, 76, 77). Combating plant disease by preventing sporulation of the causal fungi was suggested by Horsfall (40) as possibly accounting for why it is that p-dichlorobenzene, a notably poor fungicide, so successfully inhibits the blue mold fungus in tobacco seedbeds. Suggestive, too, is the work of Clayton (15) who demonstrated that oils which are not fungicidal are effective against this same fungus when applied as sprays.

Acting on the hypothesis that removal of trace metals from the medium would induce failure to form spores by the fungus, Rich and Horsfall (63) have added to nutrient medium dimethyl glyoxime, a reagent which reacts with metals in the medium, rendering them unavailable to the fungus. *Aspergillus niger* grew vegetatively to an equivalent extent on this medium but failed to produce spores.

c. Inactivating or Antidoting a Toxin Produced by the Pathogen. A number of plant pathogens produce toxic substances, either on culture media or in the diseased plant, and these are themselves primary factors in pathogenesis (14, 29, 61). Howard (45, 46) demonstrated this in the case of bleeding canker disease of hard maples. He suggested that chemicals which react with the toxin, antidoting it, should be effective in combating the disease. The dihydrochloride of diamino azobenzene was found to antidote this toxin. He was thus the first to apply the idea of toxin inactivation to disease control. In relation to Dutch elm disease, Zentmyer (99, 100) noted the existence of a toxin, and subsequently noted how the toxin behaves by itself in the tree and in combination with chemicals acting as chemotherapeutic agents (105).

d. Preventing Formation of Toxin by the Pathogen. A chemical may prevent formation of the toxin but otherwise not affect the fungus deleteriously. This mode of action has not yet been explored but on logical grounds might be presumed to be superior to inactivating an already formed toxin. The weakness underlying inactivation of a toxin lies in the fact that the fungus presumably continues to grow and produce more toxin. Unless there be a continuing supply of therapeutic agent, the fungus and toxin will in the end prevail.

e. Increasing the Resistance of the Host. Finally there is the possibility that the host itself is made more resistant to disease or even immune to it. Cases of resistance to disease or of immunity which are dependent upon chemical compounds in the plant are well known. Thus protocatechuic acid, the best known illustration, is the factor in red and yellow onions which renders them immune to onion smudge (52, 94). As another example Greathouse and Rigler (33) related resistance to *Phymatotrichum omnivorum* to the alkaloid content of the host. While these are both instances of immunity in which the active chemicals occur naturally, the point is perhaps obvious that immunity can be conferred by the presence of a chemical in the plant, however it may be put there.

METHODS OF INTRODUCING CHEMICALS INTO PLANTS

The various methods by which plants may be injected have been reviewed already (54), and for purposes of the present paper there is no need to describe techniques of injection in detail other than as they are of immediate interest here. As has been noted, chemicals have been introduced into plants in the solid state and in solutions, and the methods of injection naturally fall into these categories.

Introduction of Solids

A solid introduced into the stem may have a very low level of solubility and still be effective therapeutically. The purpose for which the compound is introduced may determine how useful it is. Thus Chandler (12) has driven zinc nails into zinc deficient trees with successful results.

It is difficult to predict how a compound will behave when it is introduced as a solid into the stem. Thus an interesting contrast in behavior between two compounds occurred some years ago in New Haven. 8-quinolinol sulfate and the corresponding benzoate in powder form were each introduced into elm trees in gelatin capsules, and the holes then plugged with oak dowelling. The following year the holes were opened and it was found that the sulfate had been completely absorbed, whereas the benzoate remained in the hole as a paste. The difference in solubility of these compounds is in the favor of the sulfate, but the benzoate is soluable to the extent of one part in 1,200 of water.

Introduction of solids into tree trunks appears to have been effective in combating disease in certain instances. Thus Horsfall and Zentmyer placed gelatin capsules containing 8-quinolinol sulfate into the trunks of maples suffering from *Verticillium* wilt and obtained a favorable response from treatment in contrast with similarly diseased trees which were not treated (42, 43, 101). A second example of such behavior is that reported recently in which commercial arborists (47) have introduced pellets of sulfanilamide into elms suffering from wetwood disease (11), caused by *Erwinia nimi-pressurialis,* and have noted markedly reduced fluxing and wilting of leaves for a period after treatment.

When solids are introduced into the stem for chemotherapeutic purposes dispersion is slow, distribution of material is relatively poor, especially if the plant is large, and many points of injection are necessary. This involves more wounding of the plant than when solutions are injected.

Wounding is definitely a factor in such work. In trees wound gums, tylose formation and extensive vascular discoloration may result from making bore holes, and such wounds provide portals of entry for wood-attacking fungi (53). Broekhuizen (5) has studied the relation of wound gums to loss of conductive function in woods.

Introduction of Liquids and Solutions

In liquid form, materials may be introduced into trees in a variety of ways, and a number of them have been quite thoroughly studied. A comparison of methods of plant injection has indicated those procedures resulting in best distribution in the plant and most rapid uptake.

a. Injection Through Bore Holes. The work of Shevyrev (72) was primarily limited to injection through bore holes into the tree. He contended vigorously that, to avoid air locking of vessels, the injection hole into the trunk must be made under water. A number of workers following him were influenced by his arguments and followed his method or a modification of it. This consisted of smoothing the bark and then fastening a metal collar around the trunk of the tree so that it was watertight. After filling the collar with the solution to be injected, he made chisel cuts into the wood under liquid. Numerous subsequent modifications of his basic procedure are reviewed by May (54), these being primarily concerned with size of reservoir and number of injection holes.

Another group of workers following Roth (68) have paid relatively little attention to whether the injection hole has been made in the air or under water. This group also noted the rapidity with which trees take up solutions from the reservoir. Apparently uptake of solutions is rapid under ideal conditions, whether precautions are taken against air lock or not.

The importance of air lock in vessels has been investigated by Dickson and Blackman (17), and their experiments are illuminating. To elucidate what happens to air bubbles which form in xylem tissues of conducting wood, they examined microscopically a number of capillary systems into which air was introduced, and noted that air bubbles disappear rapidly from any capillary structure, whether living or dead, whether derived from living matter or artificially constructed. The rapidity of disappearance of air bubbles was related to capillary diameter. This would suggest that air introduced into the xylem from holes cut into a tree dissolves quite rapidly so long as the ratio of water to air in the lumens of the cells is very large, and the capillary diameter of the vessels is small. One must conclude, therefore, that Shevyrev laid undue emphasis upon preventing air lock in introducing liquids into trees.

In connection with phenomenon of air lock, a second investigation is noteworthy. The Division of Forest Pathology of the United States Department of Agriculture[2], studying the vessel structure of elms in relation to Dutch elm disease, took movies of the rate of uptake by living trees of carbon particles and yeast cells dyed with haematoxylin. The trees were stripped of bark, fitted with a metal collar after the manner of Shevyrev, and the suspensions poured into the funnel-shaped reservoir. Immediately upon making the chisel cuts, the wood into which the fluid was absorbed became colored by the absorbed suspension, the coloration moving initially up and down the stem from the point of entry at the rate of three inches per second through conductive wood. Such entry of liquid into the stem was in response to the pressure differential between the water columns in the tree and the atmosphere outside. When the pressure differential disappears, this surge ceases. Then fluid moves into the tree in response to the tranpirational loss from the leaves, this pathway of water movement offering less resistance to flow than the pathway through the roots from the soil.

---

[2] Motion picture film on movement of dyed yeast cells and carbon particles through the vessels of American elm. This film is in the Division of Forest Pathology, United States Department of Agriculture, Plant Industry Station, Beltsville, Maryland. We are indebted to Dr. Curtis May for loaning this motion picture to us.

One may conclude from these studies that Shevyrev was correct in his contention, so far as the initial surge is concerned, but incorrect as to the factors responsible and the overall picture of liquid uptake by the plant. Airlock is not a factor, but the pressure differential is, for perhaps the first two or three minutes of absorption.

There are a number of methods for introducing liquids into trees where precautions against airlock are not taken. These have already been adequately reviewed by May (54).

The pressure applied to the reservoir of solution is a second factor determining distribution and rate of absorption of solution by wood of a living tree. The effect of this factor will be discussed in the next section, it sufficing here merely to note that pressure injection has been used by several workers with varying success (23, 64, 73). When too great pressure is used, the bark and cambium can be blown off a tree.

b. Injection Through Cut Leaves, Twigs and Roots. Reference has already been made to the work of Goff (31) who attached the cut ends of roots to a reservoir above the top of a tree. This was an early case of injection of plants through cut surfaces. Several methods have been used in addition to root injection (54, 60, 64). One method employed in investigating chemotherapy of X-disease consisted of cutting off a twig tip and attaching rubber tubing to it which led to the reservoir containing the solution (83). In this considerable uptake occurred, but the volume of material absorbed depended in no small part on the nature of the solute being absorbed. Thus in one experiment with $CaCl_2$ a volume of solution greater than the total volume of the tree was rapidly absorbed. There was no leak in the reservoir system. In this case the plant must have secreted water into the soil through the root system. When malachite green was used, only a minute volume of solution was absorbed before uptake stopped. The tree then began forming gum in large quantities, and it even oozed out into the tubing and reservoir (81). Roach (64) and Storey and Leach (86) have used this same method or modifications of it in their studies.

A third procedure has consisted of cutting the tip off a leaf or leaflet and immersing this tip in solution. The amount of vascular tissue exposed determines the volume of solution absorbed and the amount of tissue which becomes permeated by solution. Roach studied injection by such means extensively, after Muller employed it in a preliminary way. He has related the amount of leaf cut off to the amount of solution absorbed and the resulting distribution in the plant (64).

c. Absorption Through Intact Surfaces.—Muller (60) immersed intact leaves in poisonous solutions and evaluated the distribution by the pattern of phytotoxicity in the plant. This was the best method of introducing chemicals which he employed. Essentially the same method was employed by Anderssen (2).

Stoddard (82, 83), Zentmyer (104) and Dimond (18, 23) at Connecticut have extensively applied solutions of chemicals to the ground over the root system, and allowed the plant to absorb the solutions in this fashion. In the field tests in which 8-quinolinol benzoate was applied to elms inoculated with Dutch elm disease, the response of treated plants was much more pronounced than by other methods of injection (22). This indicated that distribution of chemicals absorbed through the root was superior to trunk or twig injection.

DISTRIBUTION OF MATERIALS INJECTED INTO PLANTS

a. From Root Absorption. The belief has long prevailed that when fertilizer is applied laterally to the roots of a tree, response of the leaves and branches is likewise lateral. The belief has become prevalent that there is never cross transfer of materials taken up by the root, but only movement vertically upwards in the tree, allowing for spiral grain. Roach (64) has explored the extent to which this generalization can be made and has shown that at least three types of distribution may result, depending upon the method of application:

1. If the entire root system is exposed and a single root then immersed in liquid, distribution of the material will be more or less uniform throughout the plant. This result is confirmed by the work of Goff (31).

2. If the root system be left undisturbed and fertilizer applied over one root only, or if one root be exposed and the cut end immersed in solution, the nutrients will follow the grain of the wood and appear in the portion of the tree morphologically above the absorbing root. This response has been repeatedly elicited in plants by dye experiments and by applications of fertilizer and single salt solutions.

3. The third situation follows from the two already noted. If the chemical be uniformly applied to the soil around the root system, of if the root system be immersed in the chemical, the compound will be distributed uniformly in the tree. This has been demonstrated for 8-quinolinol benzoate (20) in elm by applying ferric chloride solution to cut portions of wood throughout the tree after the chemical has been applied to the soil and absorption has occurred.

b. From Leaf Absorption, Cut or Entire. Absorption of solutions by immersion of intact leaves or through their cut surfaces results in a localized pattern of distribution (64). What primarily determines the nature of this pattern through the plant is the vascular trace anatomy of the species concerned, and one can generalize as to distribution only in these terms. It would not seem that this method of injection offers much promise for actual therapy of plant disease unless many injection points be used. But from an experimental point of view in testing chemicals for their activity, it could be a very useful technique.

c. From Branch, Twig or Borehole Injection. Reputedly distribution of chemicals is poor when they are introduced into a tree through a bore hole. Studies on seasoned coniferous woods, directed toward wood preservation (74), have indicated that the resistance offered by cells to penetration is approximately 15 times as great in the radial and tangential directions as it is longitudinally. In angiospermous woods, particularly those having very long vessels of large diameter, the resistance to flow in the tangential and radial directions is very much greater than longitudinally, probably of the order of 100 times. In the living tree the xylem walls are fully hydrated, and transpirational loss results in a pressure differential between the lumens of the vessels and the outside atmosphere. Both of these factors increase resistance to flow in a radial or tangential direction, particularly if resistance to flow is a function of pressure. Pressure injection of solutions through a bore hole is limited by the foregoing factors, most particularly by the maximum pressure which can be applied to the tree without injury to cambium and bark.

Until recently very little attention was paid to the fact that distribution of an injected solution into a tree is profoundly affected by the location of the bore hole with respect to branching, crotches and other gross morphological features of the tree. If the bore hole is placed in the Y of a crotch, solution is distributed in both branches, but if placed below one branch, the solution rises into that branch only. Roach (64) has been responsible for emphasizing this point and cites experiments in which distribution was wholly unsatisfactory, owing to its neglect, and also experiments where full allowance was made with distribution of the chemical better than when uniform root absorption occurred. These experiments were made with nutrient solutions on trees suffering from a deficiency of the supplied element. Response of the tree was the criterion of distribution.

Season of the year at which treatment occurs affects distribution of the solution injected and the amount of material absorbed (20). Root pressure is usually greater than atmospheric at the time of bud expansion, and less than atmospheric when the tree is fully foliated and in a dry soil.

The amount of moisture in the soil affects distribution of chemicals within the plant, whether solutions are introduced through bore holes or applied to the soil. Roach cites an instance in which dyes, injected into a plant growing in soil saturated near the surface, but dry deeper down, penetrated to the tips of roots in dry soil but did not penetrate very far into roots in the wet soil.

d. Ease of Distribution of Chemical Through the Plant. There remains for discussion the ease with which particular compounds may be distributed through the plant after injection has occurred.

What has been said so far assumes that a solute moves freely from one part of the plant to another. Yet certain solutes are taken out of solution by plant cells and fixed either on the cell surface or within the cell so that they cannot be distributed freely once they are within the plant. Thus copper and mercury are fixed by woody cells so that the salt never moves to a great distance from the injection point if dilute solutions are used. The behavior of iron in deficient plants and of certain dyes in this fashion is well known. Zentmyer *et al* (105), in injecting chemicals into elms for chemotherapeutic purposes against Dutch elm disease, noted that distribution, as measured by injury, tended to remain localized with certain chemicals, but with others, such as borax, redistribution occurred extensively.

CONTROL OF DISEASE BY CHEMOTHERAPY

In a study of the work of the various investigators of chemotherapy in respect to materials used and diseases treated, three things are at once evident: (*a*) the large number and variety of materials that have been used successfully, (*b*) the number of unrelated substances which have shown therapeutic activity against a single disease, and (*c*) the number of unrelated diseases against which a single material is effective. These facts are perhaps noteworthy in that they follow the pattern of the use of fungicides and conventional methods of plant disease control.

With a minimum of criteria to guide the choice of therapeutants it is understandable that a large number of materials would have been tried. From these trials have appeared a very satisfying number and variety of substances showing therapeutic activity. Notable among the more widely used and effective chemicals are such metallic compounds as salts of zinc, calcium and sodium with iron, manganese, uranium, mercury, lead, potassium and magnesium being reported as showing therapeutic activity to some degree. 8-quinolinol sulfate and benzoate, several sulfa compounds, hydroquinone, quinhydrone, benzoic acid and p-nitrophenol add to a long and heterogeneous list of active chemicals. To these can be added various gases, antibiotics and plant extracts. In fact, nearly as many therapeutic materials have been found as there have been fungicides, and with all the work that has been done on screening of fungicides the criteria for their choice are not much further developed than criteria for the choice of therapeutic materials.

Crown gall, Dutch elm disease, X-disease of peach and common blight of beans may be cited as examples of single diseases being controlled by a variety of chemicals. These diseases are doubtlessly not unique in this, but it so happens that a considerable amount of work has been done on each disease, and more therapeutants have been found for them than for other diseases.

There appears to be no record of crown gall being completely controlled by the introduction of materials into a plant by root absorption or by injection, the only successful treatments having been by application of chemicals directly into or onto the gall tissue. It could be postulated that this is because infection is localized and because it is not possible to maintain a sufficient concentration of therapeutant at the point of infection. Verona (93) reported a reduction in size of galls on *Ricinus* by applications of sulfur to the soil, and with calcium, sodium, potassium and magnesium sulfate to a lesser degree, but there is no evidence that gall formation was prevented or that the gall tissue was killed. Ark in 1941 (3) successfully treated crown gall on almond by applications of either sodium dinitroorthocresol, iodine or clove oil combined with methyl alcohol, glacial acetic acid and glycerine. It appears that the latter materials were considered as adjuvants and not as therapeutants.

Brown and Boyle (7, 8) introduced crude penicillin into galls on *Bryophyllum* sp. by needle punctures, causing the death of the gall tissue without injury to adjacent healthy tissue. Brown later reported (6) the same results with both crude penicillin and streptomycin. In this work it appears that the materials affected the diseased tissue only and had no effect on healthy tissue. The effect on the causal organism was not noted. It is interesting to note that Lewis (51) found that purified penicillin did not arrest sarcoma tumors in mice, or kill sarcoma tissue in culture, but a less purified salt did kill sarcoma tissue in culture without injury to healthy tissue. Galls on various herbaceous and woody plants were successfully treated by Hampton (36) with both commercial and crude penicillin and streptomycin, the latter material being considered the more effective. In these experiments galls on various parts of the plant were treated and by various methods of application of the therapeutants with no differences in the results obtained. As with the work of others on crown gall, these results were obtained by applications of the therapeutant directly on or into the gall and not from injection or root absorption by the plant.

Common blight of beans is also an example of a disease controlled by several unrelated chemicals. The use of systemic infection of *Xanthomonas phaseoli* as a screen for therapeutants (25) showed that soil applications of salycilic acid, zinc sulfate, calcium chloride and auramine effectively controlled this disease in the systemic form. Salycilic acid was much the best chemical used in this work. It prevented symptom expression on 86% of the plants, and it was not possible to recover the organism from nearly 50% of these plants.

Hydroquinone, benzoic acid, 8-quinolinol benzoate and 8-quinolinol sulfate may be cited as examples of chemicals that have been found to be active against more than one disease. Hydroquinone has shown therapeutic activity against Dutch elm disease (105), X-disease of peach (83) and systemic infection of *Xanthomonas phaseoli* of beans (25). Benzoic acid was found to give control of X-disease of peach and Dutch elm disease (79, 105). 8-quinolinol sulfate has been shown to be effective against Dutch elm disease (21, 27, 105), X-disease of peach (79), *Fusarium* wilt of carnations (28) and *Verticillium* wilt of maple (42, 43, 101). 8-quinolinol benzoate has been found to control *Fusarium* wilt of tomato (24) as well as Dutch elm disease (21, 105) and X-disease of peach (83). It will be noted that the diseases which were controlled by the above mentioned chemicals are all vascular diseases, but there is no surety that these chemicals can be taken as criteria to predict types of chemicals which will be generally active against vascular diseases. The circumstance of fungus, bacterial and virus diseases being controlled by the same chemicals was the basis of the hypothesis (85) that the ultimate reaction of the plant to the different diseases may be the same.

There are but few foliage diseases that have been controlled by chemotherapy.This is probably true for two reasons, first, because such diseases are ordinarily well controlled by spraying and the necessity for finding other methods of control is not great; secondly, they appear at the moment to be more difficult to control by chemotherapy. Roach (65) found that injections of sodium thiosulfate into apple trees gave good control of mildew on the foliage. It is of interest to note that injection made in the fall controlled the mildew the following season better than injections made during the growing season. In the work of Demaree (16) on zinc sulfate to correct zinc deficiency in pecan trees it was found that this chemical either applied to the soil or injected into the tree through bore holes controlled *Cladosporium effusum* and *Mycosphaerella dendroides* on the foliage. Blue mold of tobacco has been controlled commercially by benzol gas (95). Wolf *et al* (95) have postulated that this gas was absorbed by the leaf tissue either directly as gas or as an aqueous solution from moisture on the leaf surface. It is also possible that the gas acted as an antisporulant and thus prevented spread of the disease. Of interest, too, is the work of Leben and Keitt who have isolated an antibiotic substance from an Actinomycete which is effective *in vitro* against *Venturia inaequalis* and in the greenhouse on apple leaves when applied as a foliage spray to prevent apple scab infection (49, 50).

Besides the bacterial diseases already noted there are a few others that have been controlled by therapeutic chemicals. Brown and Heep (9) found that plum budwood infected with *Phytomonas pruni* when treated with streptomycin under negative pressure did not transmit the disease when budded into healthy trees and also that the organism could not be recovered in culture from the treated wood. Van Schaak (92) treated potato tubers inoculated with the ring rot organism, *Corynebacterium sepedonicum*, with crude penicillin and streptomycin. The tubers treated with penicillin failed to grow, possibly because of the phytotoxicity of the material, but those treated with streptomycin made normal growth and produced tubers free from the ring rot organism. It cannot be generalized, however, that streptomycin is a specific for bacterial plant diseases, for it has been found that (1) while bean plants readily absorb this antibiotic, the presence of it in the plant has no effect on the organism when the plants are inoculated with *Xanthomonas phaseoli.* Rudolph (69) inactivated *Xanthomonas juglandis* and *Erwinia amylyvora* in culture with penicillin, but when this material was injected into mature walnut and pear trees with natural infections of the organisms it did not show therapeutic activity. From the author's discussion it appears that this failure may have been due to the conditions of the experiment rather than to lack of therapeutic activity of penicillin. Wet wood of elm has been studied in much detail by Carter (11) who found that the causal organism, *Erwinia nimipressuralis,* was present in the heartwood of the infected trees. Because of the fact that solutions are not conducted through heartwood, injections of mercuric chloride, copper sulfate, silver nitrate, 8-quinolinol sulfate and Helione did not have any effect on the organism. In a small scale experiment McClintock (56) applied high concentrations of zinc chloride to fire-blight cankers on apple which arrested further development of the original cankers and prevented secondary infections the following season. The author did not report attempts to culture the organism from the treated cankers, and no data are presented on the behavior of check trees.

Howard (45, 46) has offered the hypothesis that the disease symptoms caused by *Phytopathora cactorum* in maple are the result of the production of toxins by the organism. He postulates that injections of "Helione Orange" antidote the toxins and inhibit the growth of the causal fungus, thus producing the resultant control of the disease.

In 1903 Mokrzecki (58, 59) reported successful results in the control of anthracnose of grape with injections of iron sulfate. In this same report unspecified fungus diseases of apple and oak were said to have been controlled by the same chemical. While not within the scope of this paper it may be noted that this report indicates that the author covered a wide range of deficiency diseases and insect pests in his investigation of internal therapy.

Internal therapy for the control of verticillium wilt of hard maple was studied by Tehon and Jacobs (91) who found that injections of various chemicals gave generally unsatisfactory results. They also tried extracts of the causal fungus which were not effective. Later Tehon (90) injected American elm trees with filtered extracts of *Verticillium albo-atrum* cultures and found that the trees were not affected by subsequent inoculations of the same fungus. Apparently this extract acted directly on the fungus, since it could not be recovered from the trees which had been injected.

Probable therapeutic action is indicated in some cases by the application of materials to the surfaces of plant material or on fruiting bodies of fungi, as is shown in the following reports of several investigators. Klotz (48) has shown that nitrogen chloride considerably reduced the amount of stored orange decay. Strong and Cation (87) found that sodium dinitrocresylate sprayed on the fruiting bodies of several species of *Gymnosporangium* prevented germination of the teliospores. Yarwood (98) reported the killing of spore postules of bean rust, *Uromyces* *phaseoli typica,* by cyanide and hydrogen sulfide gas, vapor of dilute lime-sulfur and by lime-sulfur sprays, and Stoddard and Heuberger (84) showed that tetramethyl thiuramdisulfide and ferric dimethyl dithiocarbamate killed spores of carnation rust and prevented the subsequent development of spores in the sprayed pustules. These results indicate absorption of the several materials and consequent local inactivation of the fungus.

Various plant extracts have been found to inhibit the growth of fungi *in vitro.* The use of such substances as therapeutants presents a field of investigation which has not been extensively explored. Greathouse and Rigler (33) studied the effect of a large number of plant alkaloids on the growth of *Phymatotrichum omnivorum* in culture and found a high correlation between their toxicity to the fungus and the resistance rating of the plants from which they were isolated. A high percentage of a large number of plant extracts was found by Gilliver (30) to completely inhibit the germination of conidia of *Venturia inaequalis.* Notable among these extracts was that of *Hedera helix* which was active at relatively low concentrations, and the dried leaves retained their potency for as long as three months. Link and Walker (52, 94) demonstrated that colored onions are resistant to smudge, *Colletotricum circinans,* because of the presence of protocatechuic acid. This constituent is lacking in the white onion which is susceptible to smudge.

CHEMOTHERAPEUTIC STUDIES ON DUTCH ELM DISEASE

Historical. In Europe and the Americas, Dutch elm disease is of only recent importance. It was first noted in Europe after the first World War, and early investigations were directed toward finding the cause of the disease and the susceptibilities of different species and varieties of elm. It was not until 1935 that Boudru published results of tests in which malachite green and 8-quinolinol compounds were taken up by the tree (4). His conclusion was that while such techniques might prove useful, the best means of attacking the disease lay in developing resistant varieties. Not being discouraged by such a report Fron reported the following year the result of tests conducted with 8-quinolinol sulfate and claimed control of Dutch elm disease when the chemical was injected in strengths of 1 in 20,000, or when roots of trees were immersed for six hours in such solutions, or when trees were watered at the rate of 10 liters of chemical per tree, treatment being given every two weeks during the growing season. (27). In view of what has been learned since, there is little doubt that Fron's observations were correct in quality, but it is likely that the reason that nothing further was heard from these investigations lies in the fact that the solutions were very dilute, the quantities applied to the tree were very small, and the results which he reported were based on 17 trees only.

Hypotheses on Modes of Action. A factor in the undertaking of chemotherapeutic work on Dutch elm disease at the Connecticut Station was the research done by Howard during 1940 and published in 1941 on bleeding canker of maple (45, 46). In publishing the results of his work, Howard demonstrated that *Phytophthora cactorum* forms toxins in the maple, and that these toxins can be neutralized or counteracted by the dihydrochloride of di-amino azo benzene, a dyestuff sold under the trade name of Helione Orange. Caroselli carried this work forward, and it is presently the basis of a patent for treatment of certain vascular diseases.

Work was thus begun at the Connecticut Agricultural Experiment Station by Zentmyer and Horsfall, and quite soon after its inception, toxins were isolated from cultures of *Ceratostomella ulmi,* which upon injection into a healthy tree reproduced at least a part of the symptom picture of Dutch elm disease (99, 100).

These workers recognized the possibilities of controlling Dutch elm disease by chemotherapeutic means, and particularly emphasized toxin inactivation by chemicals injected into the sick tree as a possible means of attack (42). Early experimental evidence indicated that urea, the dihydrochloride of diaminoazobenzene, and 8-quinolinol sulfate would inactivate the toxic fractions isolated from *Ceratostomella.* The fact was pursued by injecting these chemicals into trees inoculated with Dutch elm disease and trees naturally infected with the disease. Preliminary results indicated favorable response on the part of the trees (101, 105).

Subsequent work on the nature of the toxins has indicated that several toxins occur, each being responsible for its portion of the symptom picture (19, 22). From metabolism solutions of *Ceratostomella,* a carbohydrate fraction has been isolated that causes response in tomato and elm cuttings almost identical with those described (38, 39) as being formed by the crown gall organism in its cultures. This carbohydrate fraction is probably not a simple polysaccharide, since pectic enzymes partially break it down, as also do amylase type enzymes, but acid hydrolysis strikingly increases the reducing sugar present, indicating carbohydrate nature. A second fraction, responsible for necrotic spotting of leaves, has also been isolated from culture solutions.

The above data are pertinent in that tests with these fractions, either combined or in more pure condition, have never indicated toxin inactivation by the chemicals earlier reported as reducing toxin activity, and it is difficult to understand how such inactivation could be accomplished by these compounds upon a uronic acid type of polysaccharide or pectin.

In view of these results and for reasons outlined in an earlier section of the present paper, more recent trends in chemotherapy of Dutch elm disease, at least in Connecticut, have been away from simple toxin inactivation.

The nutritional requirements of *Ceratostomella ulmi* have come in for scrutiny, and the fact that *C. ulmi* cannot synthesise pyridoxine but must be supplied it has been noted (10, 67). Recent developments in the field of animal chemotherapy indicated that a possible mode of activity of sulfa compounds lies in the fact that these molecules closely resemble essential metabolites, both structurally and chemically (96). According to these ideas, an organism might be "stymied" by being subjected to compounds resembling essential metabolites but forming irreversible union with the enzyme instead of a reversible union as a part of a metabolic cycle. Woolley (97) has extended this idea strongly in the field of medicinal chemotherapy and of biochemistry, and Zentmyer clearly stated that use could be made of these lines of reasoning in the field of plant chemotherapy (101). To date it would seem that this promising approach to chemotherapy of Dutch elm disease has borne little fruit, but has likewise been explored far too little.

Yet another method of attacking the fungus chemotherapeutically was suggested by Zentmyer (102, 103) and elaborated by Horsfall and Zentmyer (44). The results of preliminary trials with chemicals indicated quite clearly that 8-quinolinol sulfate was effective to some degree as a chemotherapeutic agent against Dutch elm disease. Incidentally this series of trials of 8-quinolinol and its derivatives arose independently and quite without knowledge at the time of the French and Belgian work cited earlier. It occurred to Zentmyer that, inasmuch as these compounds are all used in quantitative chemical analysis for detecting a number of metallic cations, a possible mode of action against the fungus lay in its removing essential metals from the substrate. While Cifferi and Baldacci (13) have questioned whether this hypothesis accounts for all of the activity of such compounds, *e.g.*, in the case of zinc salts, the experiments published by Zentmyer detailed convincing evidence that at least a part of the activity is in accordance with his hypothesis (102, 103).

Tests on Chemicals of a Preliminary Nature. Initially tests were made on over 100 compounds, the screening procedure being based on toxicity to the pathogen, compared with toxicity to the host (96, 105). Compounds indicating promise on this criterion were injected into a few elms of nursery size growing under field conditions. Such trees were inoculated with Dutch elm disease, and the chemical was injected as a solution into the trunk of the tree. Records were kept of phytotoxicity of materials, but most important was the record of the amount of damage done by Dutch elm disease by the end of the season in relation to treatment, and the rate at which the disease spread in the tree (106). Such work began in 1940 and has continued to the present date in modified form. The various chemicals which were employed in these tests have been detailed elsewhere in this publication, and such details may be obtained from the detailed summary of these tests (105). Suffice it to state that among the most promising chemicals were 8-quinolinol sulfate, 8-quinolinol benzoate, p-nitrophenol and hydroquinone.

Early trials with 8-quinolinol sulfate indicated a marked response by diseased trees, but the response was of short durations. While flagging and wilting tended to disappear after treatment, and spread of such symptoms was stopped, this effect wore off in about two weeks, and retreatment was necessary. Diseased trees eventually died despite treatment. Such results prompted Zentmyer, Horsfall and Wallace to seek a more effective compound, and 8-quinolinol benzoate did indeed prove to have properties superior to those of the sulfate as a chemotherapeutic agent (41, 102, 105).

In addition, more emphasis was laid on the necessity of obtaining satisfactory distribution of chemical throughout the treated tree, and Zentmyer began exploring the possibility of applying solutions to the soil rather than injecting them into the trunk (101). It remained, however, for Stoddard (84) to obtain evidence that the response of trees treated with 8-quinolinol benzoate by soil application was much superior to that of trunk injection, and from that time, soil application has been the stressed technique of application.

Results of Field Tests with 8-quinolinol Benzoate. Field experiments, using large elms, on the effect of 8-quinolinol benzoate were begun in 1946, which indicated with high statistical significance that trees heavily inoculated with *Ceratostomella ulmi* resist the disease to a greater extent when treated than if left untreated (18, 21, 22, 41). The chemical was applied to the soil as a solution over the feeding root area. As a result of treatment, trees were less extensively invaded by disease, and held their leaves later in the season, although all trees became diseased when the method of inoculation was very severe.

When the compound is applied as a solution to the soil surface and allowed to soak into the soil it is taken up into the tree. The effect of treatment lasts from 90 days to as long as one growing season, but the beneficial effect wears off after one year (21, 23). Three methods of applying OQB to the soil were compared. These consisted of (*a*) scattering the dry chemical around the base of the tree over the feeding root area and watering it in, (*b*) watering solutions on the surface of the soil, and (*c*) injecting solutions beneath the surface of the soil under pressure. The third procedure was distinctly the best of the three. Trees so treated were damaged less by disease and showed less invasion by the fungus. Moreover a smaller percentage of such trees took the disease depite repeated inoculations with the fungus (21, 23).

Qualitatively it has appeared that early spring application of chemical is almost without effect on the tree, that applications made when the leaf buds are expanding reduces invasiveness of the disease and the amount of wilting and flagging of branches from disease, and that applications made during the late summer and fall decrease the extent of over-winter dieback of buds and branches (21, 23).

In a very few cases trees which were diseased when treated have recovered from the disease. This appears to happen more frequently than occurs naturally (21, 23). Incomplete evidence would also indicate that healthy treated trees do not become infected so frequently as do untreated ones (21, 23).

The possible value of bacteria which produce substances antibiotic to *Ceratostomella ulmi* is receiving critical evaluation from the chemotherapeutic point of view. Pomerleau and Lechavalier have recently noted that such compounds are formed by an unidentified but described bacterium (62). In 1946 Burkholder[8] gave to the authors three cultures of different Actinomycetes which produced substances antibiotic to this fungus. Szkolnic has also noted such antibiotic materials produced by an *Actinomyces* species. (89).

CHEMOTHERAPY OF X-DISEASE OF PEACH

The first successful inactivation of the X-disease virus was obtained in 1941 (41, 79, 83) by soaking budwood from diseased trees in aqueous solutions of chemicals. After this treatment the buds were washed in distilled water and set in healthy seedlings. Failure to transmit the disease to these seedlings was taken as the measure of inactivation of the virus. Some of the chemicals, notably quinhydrone, 8-quinolinol sulfate, hydroquinone, p-nitrophenol and calcium 8-hydroxyquinolate, inactivated the virus in many of the buds, as indicated by the consequent failure to transmit the disease. Urea, thiourea, benzoic acid and 8-quinolinol benzoate also inactivated the virus but to a lesser degree. The untreated buds and those treated with some of the chemicals readily transmitted the disease, thus indicating no inactivation of the virus. The action of 8-quinolinol sulfate was unique in that it produced a temporary inactivation of the virus, and the apparently healthy inoculated trees all showed symptoms of X-disease after a period of dormancy.

It was postulated (*a*) that the action of the chemicals was directly on the virus and not on the plant, since the amount of chemical in the treated buds seemed inadequate to affect the entire plant in which the bud was set; and (*b*) that it was not due to the decreased concentration of the virus by soaking, for buds soaked in tap water and some of the chemicals gave a high percentage of transmission.

---

[8] We are indebted to Dr. Paul R. Burkholder for calling these cultures to our attention and for giving us transfers of them.

Later experiments (80, 83) showed that the virus of X-disease could be inactivated in small seedling trees by injection or by soil applications of aqueous solutions of various chemicals. The chemicals showed no apparent lack of therapeutic activity when applied to the soil, and since this technique gave promise of successful field use, *it is currently* being used as the preferred experimental technique.

p-*aminobenzene sulfonamide* was the first chemical found to show therapy of X-disease in living plants, and later several sulfa compounds were found to have equally high therapeutic activity. It is of interest to note that p-aminobenzene sulfonamide did not inactivate the virus in diseased buds which were soaked in an aqueous solution, but as high as 100 per cent control was obtained when the material was injected into the plant after inoculation. In addition to the sulfa compounds several other chemicals were found to inactivate the virus. Among these were zinc sulfate, 8-quinolinol benzoate, disodium ethylene bisdithiocarbamate, maltose, dextrose, hydroquinone, lauryl isoquinolinium bromide and calcium chloride.

Extracts of the leaves and stems of wild black cherry (*Prunus serotina*) have shown considerable activity against X-disease of peach. Both alcohol and water extracts were moderately successful in preventing infection of peach trees by the X-disease virus. The wild black cherry is immune to X-disease, and it would appear that the substance producing the immunity is extractable and when introduced into peach trees produces immunity in them.

Most of the chemicals gave significantly better control when applied after inoculation of the treated plants. The notable exceptions to this were zinc sulfate, p-toluene sulfonamide and disodium ethylene bisdithiocarbamate. It is postulated that the chemicals which were effective before inoculation had an effect on the plant which prevented multiplication of the virus and that the chemicals effective after inoculation directly inactivated the virus. p-aminobenzene sulfonamide is an exception to this hypothesis, as it appears from the bud-soaking experiment that this material does not have a direct effect on the virus.

It was found that p-aminobenzoic acid was not in itself therapeutically active; it also antidoted the therapeutic activity of p-aminobenzene sulfonamide as it does in animals. Dextrose was found to have a similar antidoting effect on the activity of 8-quinolinol sulfate.

Some of the chemicals were effective up to ten months after application, as evidenced by the failure of many of the treated trees to become infected when reinoculated without further treatment. That these trees were not symptomless carriers is shown by the fact that buds from these trees set in healthy trees failed to transmit the disease.

SUMMARY

For centuries men have toyed with the idea of chemotherapy of plant diseases. Achievement in pest control is a development of the twentieth century, and Muller (60) has carefully reviewed the earlier work. The present paper reviews particularly the progress which has been made since 1930.

Intensive effort is now being made to determine what kinds of chemicals are effective and what types of diseases are controllable through the chemotherapeutic approach. In particular, virus diseases, systemic bacterial infections and the vascular wilt diseases have been found to yield to the proper chemicals when the latter are applied to the soil and the plant absorbs them through its roots.

Because the mode of action of a therapeutant governs the design of tests in which the effectiveness of chemicals is measured, a number of modes of action are discussed. These are, for virus diseases: (*a*) inactivating the virus *in vivo,* (*b*) preventing virus multiplication and (*c*) conferring immunity upon the host; for bacterial diseases: (*a*) inactivating the parasite, (*b*) preventing its reproduction, (*c*) inactivating toxins, (*d*) preventing their formation and (*e*) increasing resistance of the host.

Methods of introducing chemotherapeutants into plants are varied with respect to technique involved and adaptability to the purpose at hand. Injection through bore holes in the trunk has been used effectively by Roach (64) and others, but requires skill and a consideration of the topography of the tree if distribution of the chemical is to be adequate. Precautions against entry of air into the bore hole have apparently been overemphasized in the past. Soil watering and root absorption give uniform distribution of chemical if it is uniformly applied about the feeding roots. However, many chemicals will react with soil constituents, and the method tends to be wasteful of chemicals as a result.

This field of research offers promise as a new approach to plant disease control, if the existing literature is a guide. The technique of neutralizing the toxins of *Phytophthora cactorum* in hard maple suffering from bleeding canker by injecting diaminoazobenzene dihydrochloride (45, 46) is a case in point. Other examples are the extensive experiments on chemotherapy of X-disease of peach and of Dutch elm disease. To our knowledge the most striking results with any disease have been obtained with the common bacterial blight of beans.

For such use a wide variety of compounds has been effective for one disease or another. Sulfanilamide, quinolinol compounds, phenols have all been effective. Too little is known yet to generalize with respect to probable modes of action or likely chemical structures for any given disease.

It is significant that the technique appears to lend itself particularly to types of diseases for which control practices are very unsatisfactory at the present time: the vascular parasites and virus diseases.

LITERATURE CITED

1. Anderson, H. W., and Nienow, Inez. Effect of streptomycin on higher plants. Phytopath. **37**: 1. 1947.
2. Anderssen, F. G. Chlorosis of deciduous fruit trees due to a copper deficiency. Jour. Pom. **10**: 130–146. 1932.
3. Ark, P. A. Chemical eradication of crown gall on almond trees. Phytopath. **31**: 956–957. 1941.
4. Boudru, M. The elm disease in Belgium. Bul. Soc. For. Belg. **42**: 508–522. 1935.
5. Broekhuizen, S. Wondreaksies van Hout. Het ontstann van thyllen en wondgom in het biezonder in verband met de itpenziekte. Thesis, Univ. Utrecht. 1929.
6. Brown, J. G. Cytological effects of penicillin and streptomycin on crown gall. Phytopath. **38**: 5. 1948.
7. ———, and Boyle, Alice M. Effect of penicillin on a plant pathogen. Phytopath. **34**: 760–761. 1944.
8. ———, and ———. Penicillin treatment of crown gall. Science **100**: 528. 1944.
9. ———, and Heep, Don. Effect of streptomycin on budwood infected with *Phytomonas pruni*. Science **95**: 127–128. 1942.
10. Burkholder, P. R., and McVeigh, Ilda. Pyridoxine as a growth factor for *Graphium*. Science **95**: 127–128. 1942.
11. Carter, J. C. Wetwood of elms. Bul. Ill. Nat. Hist. Survey **23**: 407–446. 1945.
12. Chandler, W. H. *et al.* Little leaf or rosette of fruit trees. Proc. Am. Soc. Hort. Sci. **30**: 70–86. 1933.
13. Cifferi, R. and Baldacci, E. Failure of the antagonistic action of a zinc salt on the fungistatic ability of 8-hydroxyquinoline. Farmace **1**: 250–253. 1946.
14. Clauson-Kass, N. *et al.* Uber ein welkerzeugendes Stoffwechselprodukt von *Fusarium lycopersici* Sacc. Ver. Schw. Bot. Ges. **54**: 523–528. 1944.

15. Clayton, E. E. Fungicidal tests on blue mold (*Personospora tabacina*) of tobacco. Jour. Agr. Res. **66:** 261–276. 1943.
16. Demaree, J. B. Progress of Pecan rosette control. Proc. 27th Ann. Conv. Georgia-Fla. Pecan Grow. Assn. 1933.
17. Dickson, H., and Blackman, V. H. The absorption of gas bubbles present in xylem vessels. Ann. Bot. **2:** 293–299. 1938.
18. Dimond, A. E. Oxyquinoline benzoate aids suppression of symptoms of Dutch elm disease. Phytopath. **37:** 848. 1947.
19. ————. Symptoms of Dutch elm disease reproduced by toxins of *Graphium ulmi* in culture. Phytopath. **37:** 7. 1947.
20. ————. [Unpublished data]. 1947.
21. ————. Chemotherapeutic action of 8-quinolinol benzoate against Dutch elm disease. Phytopath. **38:** 9. 1948.
22. ————, *et al.* Chemotherapy of Dutch elm disease. Trees **7:** 6–16. 1947.
23. ————, *et al.* Progress in the chemotherapy of Dutch elm disease. Proc. 23rd. Nat. Shade Tree Conf. **23:** 1947.
24. ————, and Stoddard. E. M. [Unpublished data]. 1947.
25. ————, and ————. Common blight of beans as a screen for testing chemotherapeutic activity. Phytopath. **38:** 1947.
26. Flint, W. P. The effect of cyanide of potassium on the locust borer and the locust tree. Science **42:** 726. 1915.
27. Fron, G. The elm disease. Comp. Rend. Acad. Agr. France **22:** 1081-1089. 1936.
28. ————. Le maladie de la fusariose des Ouillets. Rev. Path. Veg. **23:** 131–144. 1936.
29. Gaumann, Ernst and Jaag, Otto. Die physiologischen Grundlagen der parasitogenen Welkens. I. Ber. Schw. Bot. Ges. **57:** 1–34. 1947.
30. Gilliver K. The effect of plant extracts on the germination of conidia of *Venturia inaequalis*. Ann Appl. Biol. **34:** 136–143. 1947.
31. Goff, E.S. The application of artificial root pressure on recently transplanted trees. Wis. Agr. Exp. Sta., Ann. Rep. **14:** 272–282. 1897.
32. Goppelsroeder, F. Cappillar-analyse berühend auf Capillaritats und Adsorptions-erscheinungun mit dem Schlusskapitel: das Emporsteigen der Farbstoffe in den Pflanzen. 545 pp. 1901.
33. Greathouse, G. A., and Rigler, N. The chemistry of resistance of plants to *Phymatotrichum* root rot. V. Influence of alkaloids on the growth of fungi. Phytopath. **30:** 475–485. 1940.
34. Hales, S. Statical essays. Vol. 1. 376 pp. 1731.
35. Hambidge, G. *et al.* Hunger signs in crops. 327 pp. 1944.
✓36. Hampton, J. E. Cure of crown gall with antibiotics. Phytopath. **38:** 13. 1948.
37. Hartig, T. Über die endosmotischen Eigenschaften der Plasmahaute. Bot. Zeit. **11:** 309–317. 1853.
38. Hodgson, R. *et al.* Wilting induced in tomato cuttings by toxic substances from crown gall bacteria. Phytopath. **36:** 401. 1946.
39 ————. *et al.* A wilt-inducing toxic substance from crown gall bacteria. Phytopath. **37:** 301–318. 1947.
40. Horsfall, J. G. Fungicides and their action. 239 pp. 1945.
41. ————. *et al.* Chemotherapy for plant diseases. Agr. Chem. 11, No. 3. 1947.
42. ————, and Zentmyer, G. A., Jr. Chemotherapy for vascular diseases of trees. Proc. 17th Nat. Shade Tree Conf. **17:** 7–15. 1941.
43. ————, and————. Antidoting the toxins of plant diseases. Phytopath. **32:** 22–23. 1942.
44. ————, and ————. Fungicidal action of reagents for amino acids, amines, aldehydes, and other reactive cell constituents. Phytopath. **34:** 1004. 1944.

45. Howard, F. L. Antidoting of toxins of *Phytoppthora cactorum* as a means of plant disease control. Science **94**: 345. 1941.
46. ———. The bleeding canker disease of hardwoods and possibilities of control. Proc. 8th. Western Shade Tree Conf. 1941.
47. Irish, Chas. F. Report on treatment of elm to control slime flux. Trees **7**: 11. 1947.
48. Klotz, L. J. The use of nitrogen trichloride and other gases as fungicides. Phytopath. **24**: 11–41. 1934.
49. Leben, C. and Keitt, G. W. The effect of an antibiotic substance on apple leaf infection by *Venturia inaequalis*. Phytopath. **37**: 14. 1947.
50. ———, and ———. Greenhouse tests of an antibiotic substance as a protectant spray. Phytopath. **38**: 18. 1948.
51. Lewis, Margaret Reed. The failure of purified penicillin to retard the growth of grafts of sarcoma in mice. Science **100**: 314–315. 1944.
52. Link, K. P. *et al.* The isolation of protocatechuic acid from pigmented onion scales and its significance in relation to disease resistance in onions. Jour. Biol. Chem. **81**: 369-375. 1929.
53. Lorenz, R. C. Discoloration and decay resulting from increment borings in hardwood. Jour. For. **42**: 37–43. 1944.
54. ———. Methods of tree injection. Trees **4**: 7–16. 1941.
55. McCallan, S. E. A. Outstanding diseases of agricultural crops and uses of fungicides in the U. S. A. Contr. Boyce Thomps. Inst. **14**: 105–116. 1946. ,
56. McClintock, J.A. The relation of canker treatment to fire blight control. Phytopath. **21**: 901–906. 1931.
57. McNab, W. R. Experiments on the movements of water in plants. Trans. Royal Irish Acad. **25**: 343-369; 567–579. 1875.
58. Mokrzecki, S. New method of healing and nourishing trees. [Abs. in Exp. Sta. Rec. **16**: 982. 19].
59. ———. Über die innere Therapie der Pflanzen. Zeit. Pflanzenk. **13**: 257–265. 1903.
60. Muller, A. Die innere Therapie der Pflanzen, Monographien der angewandten Entomologie. No. 8. 1926.
61. Plattner, Pl. A., and Clauson-Kaas, N. Über Lycomarasmin, der Welkstoff aus *Fusarium lycopersici*...Experientia **1**: (6). 1945.
62. Pomerleau, R., and Lechavalier, H. Étude de l'effet antibiotique d'une bacteria sur le developpement du *Ceratostomella ulmi* (Schwarz.) Buisman. Revue Canadienne Biologie **6**: 478–484. 1947.
63. Rich, S., and Horsfall, J. G. Metal reagents as antisporulants. Phytopath. **38**: 22. 1948.
64. Roach, W. A. Plant injection as a physiological method. Ann. Bot. **3**: 155–226. 1939.
65. ———. Injection for the diagnosis and cure of physiological disorders of fruit trees. Ann. Appl. Biol. **21**: 333. 1934.
66. ———. Plant injection for diagnostic and curative purposes. Imp. Bur. Hort., Pl. Crops, Tech. Comm. 10. E. Malling Res. Sta. 78 pp. 1938.
67. Robbins, W. J., and Ma, Roberta. Specificity of pyridoxine for *Ceratostomella ulmi*. Bul. Torrey Bot. Club. **69**: 342–352. 1942.
68. Roth, C. Eine Methode der kunstlichen Baumernahrung. Chem. Zeit. **20**: 344–345. 1896.
69. Rudolph, B. A. Attempts to control bacterial blights of pear and walnut with penicillin. Phytopath. **36**: 717–725. 1946.
70 Sachs. J. Ein Beitrag zur Kenntnis des aufsteigenden Saftstroms in transpirenden Pflanzen. Arb. Bot. Inst. Wurzburg **2**: 148–184. 1878.

71. ———. Das Eisen und die Chlorose der Pflanzen. Naturw. Rdsch. 1: 257–259. 1886.

72. Shevyrev, I. Extraradicate nutrition of diseased trees. Bot. Div., St. Petersburg Imp. Soc. Nat. Conf. 1894.

73. Southwick, R. W. Pressure injection of iron citrate into citrus trees. Proc. Am. Soc. Hort. Sci. **46**: 27–31. 1945.

74. Stamm, A. J. Passage of liquids, vapors and dissolved materials through softwoods. U. S. Dept. Agr., Tech. Bul. 929. 1946.

75. Steingerg, R. A. A study of some factors in the chemical stimulation of the growth of *Aspergillus niger*. Am. Jour. Bot. **6**: 330–372. 1919.

76. ———. The so-called "chemical stimulation" of *Aspergillus niger* by iron, zinc and other heavy metal poisons. Bull. Torrey Bot. Club. **61**: 241-248. 1934.

77. ———. Relation of carbon nutrition to trace element and accessory requirements of *Aspergillus niger*. Jour. Agr. Res. **59**: 749–764. 1939.

78 ———. Action of some organic compounds on yield, sporulation and starch formation of *Aspergillus niger*. Jour. Agr. Res. **60**: 765–773. 1940.

79. Stoddard, E. M. Inactivating *in vivo* the virus of X-disease of peach by chemotherapy. Phytopath. **32**: 17. 1942.

80. ———. Immunization of peach trees to X-disease by chemotherapy. Phytopath. **34**: 1011. 1944.

81 ———. [Unpublished data]. 1944.

82. ———. Soil applications of oxyquinoline benzoate for control of foliage wilting in elms caused by *Graphium ulmi*. Phytopath. **36**: 682. 1946.

83. ———. X-disease of peach and its chemotherapy. Conn. Agr. Exp. Sta., Bul. 506. 1947.

84. ———, and Heuberger, J. W. Eradicant action of fungicides on spores on living plants. Phytopath. **33**: 1190. 1943.

85. ———, and Zentmyer, G. A., Jr. Possible similarity of virus and fungus toxins. Phytopath. **33**: 20. 1943.

86. Storey, H. H., and Leach, R. A sulphur deficiency disease of the tea bush. Ann. Appl. Biol. **20**: 23–55. 1933.

87. Strong, F. C., and Cation, D. Control of cedar rust with sodium dinitrocresylate. Phytopath. **30**: 983. 1940.

88. Surface, A. Cyanide of potassium in trees. Science **40**: 852. 1914.

89. Szkolnic, M. Antagonistic activity of a species of *Actinomyces* against *Ceratostomella ulmi*. Phytopath. **38**: 85–87. 1948.

89a. Takahashi, W. N. The inhibition of virus increase by malachite green. Science **107**: 226. 1948.

90. Tehon, L. R. Experimental immunization of American elm from infection by *Verticillium albo-atrum*. Phytopath. **34**: 1012. 1944.

91. ———, and Jacobs, H. L. Experimental injection of hard maple saplings artificially infected with *Verticillium albo-atrum*. Davey Tree Exp. Co., Bul. 7. 1939.

92. Van Schaak, V. Antibiotics and potato ring rot. Phytopath. **38**: 29. 1948.

93. Verona, O. The repressive effect on the development of tumors due to *Bacterium tumefaciens* of sulfur applied to soil. Ann. Fac. Agr. Pisa. **6**: (7). 1945.

94. Walker, J. C., and Link, K. P. Toxicity of phenolic compounds to certain onion bulb parasites. Bot. Gaz. **96**: 468–484. 1935.

95. Wolf, F. A. *et al.* Volatile fungicides, benzol and related compounds and the principles involved in their use. Phytopath. **30**: 213-226. 1940.

96. Woods, D. D. The relation of p-amino benzoic acid to the mechanism of the action of sulphanilimide. Brit. Jour. Exp. Path. **21:** 74–90. 1940.
97. Woolley, D. W. Some new aspects of the relationship of chemical structure to biological activity. Science **100:** 579–583. 1944.
98. Yarwood, C. E. Therapeutic treatments for bean rust. Phytopath. **37:** 24. 1947.
99. Zentmyer, G. A., Jr. Toxin formation by *Ceratostomella ulmi.* Science **95:** 512–513. 1942.
100. ————. Toxin formation and chemotherapy in relation to Dutch elm Disease. Phytopath. **32:** 20. 1942.
101. ————. Vascular chemotherapy. Trees **6:** 7–17. 1943.
102. ————. Mechanism of action of 8-hydroxyquinoline. Phytopath. **33:** 1121. 1943.
103. ————Inhibition of metal catalysis as a fungistatic mechanism. Science **100:** 294–295. 1944.
104. ————, Horsfall, J. G. Internal therapy with organic chemicals in treatment of vascular diseases. Phytopath. **33:** 16–17. 1943.
105. ———— *et al.* Dutch elm disease and its chemotherapy. Conn. Agr. Exp. Sta., Bul. 498. 1946.
106. ———— *et al.* New research on the Dutch elm disease. Proc. 20th. Nat. Shade Tree Conf. **20:** 115–119. 1944.

BREEDING FOR RESISTANCE TO FUSARIUM AND VERTICILLIUM WILTS

C. D. SHERBAKOFF
Tennessee Agricultural Experiment Station

INTRODUCTION

Breeding of plants for disease resistance began toward the end of the nineteenth century with the development of Fusarium wilt-resistant Sea-Island cotton by the growers on the Sea Islands of South Carolina (117). Shortly thereafter appeared the first reports of Fusarium wilt of different plants, by Atkinson (6), Smith (147, 149), Galloway (53), Bolley (19), Orton (113) and others; and Orton (112) started experimental breeding for wilt resistance. Since then, breeding for resistance to Fusarium wilt has been extensive and in some instances remarkably successful. Resistant varieties of aster, cabbage, celery, cotton, flax, pea, sweetpotato, tomato and watermelon are grown now over large areas of cropland where the susceptible varieties formerly cultivated will no longer thrive.

In spite of the great number of plants affected and the damage caused by Verticillium wilt, there has been relatively little—though some very successful (105)—breeding for resistance to this disease. The work did not seem to have much promise of success, because of the apparent ability of the pathogen to affect a great number of unrelated hosts, and until lately the wilt was confused with Fusarium wilt (94).

THE PATHOGENS

All Fusaria causing wilt of plants, with some uncertain exceptions, are members of section Elegans (202). They are characterized by sickle-shaped, usually three-septate macroconidia, apically attenuated; by oval to kidney-shaped, mostly non-septate microconidia; by a mycelium which is white or pink, lilac or rose on acid and blue on alkaline medium; and by terminal and intercalary chlamydospores. Since there appear to be no reliable morphologic differences between most of the known pathogens of section Elegans, and because these organisms are greatly affected by environment, Snyder and Hanson (156) "reduced all former species in section Elegans to synonymy with *F. oxysporum* (Schl.) Snyder and Hansen, with *'forma'* [Snyder and Hansen] name attached to that of the species to indicate relative specificity for host as well as pathogenicity". Antedating the Snyder and Hansen proposal, Nelson, Coons and Cochran (108) came to a like conclusion in regard to the different species of Orthoceras subsection of section Elegans. They believed, however, that because of the importance of the host specificity, the species names of these Fusaria, whenever feasible, should be indicative of the host, allowing the use of the the *forma* name to indicate cultural or disease-symptom differences, as in the case of the Fusarium wilt of celery. It seems that it would be a more workable nomenclature if all species and variety names of the genus *Fusarium* well known in the literature or designating a specific pathogenicity were retained with the understanding that there may be no dependable morphologic differences between them.

In 1935 Wollenweber and Reinking (202) published a monograph of the genus *Fusarium,* with a review of all plant diseases known to be caused by or associated with *Fusarium.* They name 25 species of crop plants with varieties resistant to Fusarium wilt or to tuber, stem, root or bulb rot.

Verticillia causing wilt are Hyphomycetes, producing oval, hyaline, non-septate conidia, borne on the tips of sterigmas, arranged in a whorl or series of whorls. The first report of a wilt due to *Verticillium,* that of potatoes, was made in 1879 by Reinke and Berthold (128) who described the fungus and named it *V. albo-atrum.* Since then and up to 1929, according to Rudolph (134) the disease has been reported definitely in over 120 species of 35 widely unrelated families and 18 orders. Later the disease was reported in some other plants. In several instances the pathogen involved was described as a new species. Carpenter (27), Rudolph (134), Wollenweber (201) and some other workers consider that, in the words of Rudolph, "in most instances the claim of these organisms to specific rank is exceedingly doubtful. The most conspicuous of these is *V. dahliae* Klebahn (81). Evidence is advanced to show that this fungus is not entitled to specific rank". Therefore, new name combinations had been proposed with the different pathogenic Verticillia as new varieties of *V. albo-atrum* R. & B. On the other hand, Berkeley, Madden and Wilson (12), Ludbrook (91), Hubert and Jones (64), van Beyma (175) and some others consider the species valid because the difference between the *V. albo-atrum* R. & B. and *V. dahliae* Kleb.—the first one with dark "resting mycelium" and the other with "pesudo-sclerotia" (12)—is clearly recognizable and because in Reinke and Berthold's paper (128) there is no direct evidence that their fungus produces sclerotia of the same kind as those found in *V. dahliae* Kleb.

Host specificity of Fusaria causing wilt is generally reported. However, there are indications (2, 153) that some of the Fusaria may be parasitic on more than one species of host. On the other hand, *Verticillium* is considered by Rudolph (134) to be "peculiar in that it attacks a large number of widely unrelated plants". However, Nelson (106) found that the Verticillium of mint wilt is definitely specific in its pathogenicity, besides being different in its higher optimum temperature; and Verona and Ceccarelli (178) reported the optimum pH for *V. albo-atrum* to be 8.5, and for *V. dahliae* only 4.9, with other cultures intermediate.

Biological specialization has not been definitely shown either in *Fusarium* or in *Verticillium* causing wilt, though this is indicated for both fungi (8, 24, 101, 140, 177). There seems, however, to be a marked difference in virulence of the different isolates of the pathogens.

THE DISEASES

In Fusarium wilt the leaves usually turn yellow and die progressively from the lowest upward without any conspicuous defoliation; there are dark discoloration of the vascular system of the stem and root, wilting of the plant tops, and sometimes, as in cabbage and tobacco, more or less definite bending of the stems toward the more severely affected side. The disease may kill the plants at any stage, depending on environment, load of inoculum and other factors.

Verticillium wilt in certain cases can be distinguished from Fusarium wilt, for example, by lighter-green to yellow spotting and conspicuous shedding of the leaves of cotton and by a bluish color of raspberry canes. Usually, however, only isolation of the parasite from the diseased plant can be depended upon for identification of the disease. There is no difficulty in separating *Verticillium* from *Fusarium* in cultures.

Testing of resistance to Fusarium or Verticillium wilt is usually conducted in a field in which the host has been previously attacked by the disease. It is the only method that can be used when no dependable technique for artificial inoculation is available and when the practical aspects of the resistance obtained must be determined. The method, however, has great disadvantages: as a rule the disease in the field has an uneven appearance; usually only one generation of test plants can be tested during an entire year; and the disease may not develop sufficiently if the weather happens to be unfavorable for it. Whenever feasible, therefore, the breeder depends largely on artificial inoculation. Direct introduction of the culture into a wound of stem or root has usually been unsuccessful (134), whereas proper introduction of the fungus into soil or other medium for plant culture as a rule has been successful (2, 134, 142). The success of inoculation through the soil appears to be due to the fact that the parasite's effective entrance into the host is through the parts of the roots exposed to the invasion, as shown by Wilson (200). It seems clear that more rapid progress in breeding can be expected when an effective technique for artificial testing of resistance is employed.

THE BREEDING OF DIFFERENT CROPS

Aster

Fusarium wilt, or stem rot, of China aster (*Callistephus chinensis* Nees) is widespread in the United States and Canada and has been reported (202) from Australia, Europe, Japan, New Zealand, South Africa and Tasmania. In 1918 Beach (10) accurately described the disease and determined its cause, *F. conglutinans* Wr. v. *callistephi* n. v. In 1927 Jackson (66) listed this and four other strains of *Fusarium* as pathogenic to the aster. Riker and Jones (132) reported that a number of Fusaria, classified on a morphologic basis (202) as different formae, varieties, species and even sections, were of the same pathogenicity as that of *F. conglutinans* v. *callistephi* Beach. Shortly after, Riker (131) stated that one of the cultures, that of *F. fructigenum,* when checked for purity was found to be a mixture of that fungus and the pathogen. No checking of purity of the other cultures has been reported.

Jones and Riker (74) by repeated selections obtained asters fairly stabilized to resistance and of several basic colors and of a fairly wide range in flower type and growth habit. Later Riker and Jones (133) reported that the resistant strains were found to be resistant also in other parts of the world, though in Indiana they were susceptible to a local strain of the fungus. No complete resistance was found in any of the resistant lines. In 1939 a wilt-resistant China aster was reported from Germany (130).

Banana

In 1910 Smith (151) isolated a form of *Fusarium* from a specimen of banana received from Cuba and displaying banana wilt, or Panama disease. He named it *F. cubense.* In 1916 Brandes (23) reported that the *Fusarium* causing banana wilt in Porto Rico is indistinguishable from *F. cubense* Smith and gave an amplified description of the fungus.

Apparently the earliest report of the disease was made by Higgins (63) from Hawaii in 1904. Wollenweber and Reinking (202) reported its present occurrence in the entire banana-producing world wherever the pathogen is present and susceptible varieties are grown.

Brandes (22) found that this disease attacks most severely, and sometimes exclusively, the variety most widely planted, and that the varieties which are not attacked or are only slightly affected in a given locality may suffer severely in some other locality, suggesting biological specialization of the pathogen. However, some varieties, *e.g.,* Bungulan, Lacatan and Congo, appear to be generally wilt-resistant, as found by Ocfema and Palo (110) and others. The most important commercial variety in the Americas, Gros Michel, is very susceptible to the wilt.

In spite of the sterility of edible varieties, some successful hybridization has been achieved in Jamaica (152) and Trinidad (31) with Gros Michel as the female parent.

According to Cheema and Bhat (30), the breeding of wilt-resistant bananas at the Imperial College of Tropical Agriculture at Trinidad was started in 1922 by Mason and Dash. They intercrossed several edible varieties and crossed the edible varieties with fertile pollen of seed-bearing types of *Musa ornata* and *M. malaccensis,* obtaining 49 perfect seeds in Gros Michel, *M. sapientum;* 74 in Silk with pollen of the seeded types; and one seed from a cross of Gros Michel by another edible variety, Congo. In 1923 Harland

and Cheesman took over the breeding work. They obtained some seeds of the Gros Michel, 17 of which germinated. Of the five seedlings brought to fruiting from them, one of the seedlings, I. C. 1, in 1925 produced a fair-sized seedless fruit of good flavor and was resistant to wilt under severe conditions of the disease during the six-year test. After several unpromising attempts with other varieties of bananas—*M. cavendishi,* known as Cavendish, Chinese or Canary banana; and the Congo group of dwarf types—crossing and back crossing of Gros Michel were resumed in 1927 and have been continued. Back crosses of I. C. 1 and of a second immune I. C. 2 on Gros Michel gave no seed. Hence, Gros Michel was crossed with other edible varieties producing abundant pollen and with fully fertile varieties. Efforts to obtain F_2 of I. C. 1, up to 1935, gave 19 seeds that yielded three seedlings. Back crosses of I. C. 1 on its male parent gave abudant seed but no useful seedling. Two hundred seeds obtained from crosses of banana of the Fill-basket group, possessing wilt resistance and some desirable characters of Gros Michel, gave eight robust plants of promise. The breeding is conducted also with seed-producing bananas having 22 chromosomes, to evolve edible bananas which usually have 33 chromosomes.

In 1935 Wardlaw (190) published a monograph on diseases of the banana and of the Manila hemp plant.

In 1940 Uppal (170) reported that in India the Kali and Basri banana varieties—the latter a good commercial type—showed a high degree of resistance to Panama disease (Fusarium wilt) in trials in heavily infected soil near Narayangoan where the local Some variety was entirely susceptible; and in 1944 Leach (87) reported from Jamaica that a banana described as "Sai," possessing potential commercial value, is said to be immune from Panama disease and highly resistant to leaf spot (*Mycosphaerella musicola*). The variety was produced by an officer of the Agricultural Department, and the Imperial Government guaranteed prices per bunch.

CABBAGE

Cabbage yellows (*F. conglutinans* Wr.) is of common occurrence in the United States (181) and very destructive when the soil temperature during the growing season is over 20° C. (164). It occurs in Cuba and China (202), but not in Europe, and appears to be of American origin (187). All commercial cabbage varieties and strains growing in the United States except those developed for yellows resistance are susceptible to the disease (187).

In 1910, when yellows was unusually severe in Wisconsin, Jones and Gilman (73) selected about 100 healthy-looking plants in three badly cabbage-sick fields. Testing of the progenies and reselection was continued until 1915 when it was planned to grow 2,000 heads of the best yellows-resistant strain for production of seed for commercial cabbage growing in 1916 under the name Wisconsin Hollander No. 8. In 1920 Jones *et al.* (76) reported further progress in breeding yellows-resistant cabbage and other studies of the disease in Wisconsin.

Tisdale (168) found that cabbage yellows in seedlings grown in cabbage-sick soil develops in a soil temperature from 17° to 35° C. with the optimum at 26° to 29° C., the same as that for the growth of the pathogen, while the optimum for the cabbage was found to be at 20° C.; and Tims (164), using cabbage-sick soil in Wisconsin soil-temperature-control tanks (41), found that at low soil moisture and 27° to 33° C. temperature, a high percentage of plants of resistant strains of All Seasons became diseased, while at 21° to 24° C. they were affected only slightly.

Walker and Wellman (187) found various degrees of resistance in a number of European varieties: wild cabbage was highly resistant; Brussels sprouts and broccoli varieties were not seriously damaged; cauliflowers were somewhat more damaged than the two sub-species just mentioned; the kale varieties generally differed greatly in susceptibility, the Siberian kale being resistant and curly-leaf types very susceptible; kohlrabi varieties were all very susceptible; and within all of the forms tested a sufficient number of individuals survived to make possible the improvement of their resistance through selection.

The breeding of resistant strains of cruciferous vegetables has been reviewed by Walker (181). The most significant results of the studies and breeding are the development of yellows-resistant cabbage strains of all varieties important in the United States and the discovery of the presence of two types of resistance in cabbage and the related sub-species. One of these, type A, is controlled by a single gene dominant over susceptibility and does not break down at 24° C. or higher temperature; the other, type B, is controlled by a complex set of hereditary factors (181) difficult to fix and effective only at soil temperatures under 24° C. In 1944 Walker (183) reported promising results from his efforts at combining yellows resistance and mosaic resistance in Wisconsin All Seasons cabbage.

Carnation

Fusarium wilt of carnation was first reported in 1897 by Sturgis (160) in Connecticut; in 1899 Stewart (158) reported it from New York; and about the same time Delacroix (39) and Mangin (93) released their studies of the disease in France. Apparently the same disease has been reported from Belgium, Czecho-Slovakia, Denmark, England, Germany, New Zealand, South Africa and Uganda (202). In 1900 Delacroix (39) published a detailed study of the wilt and named the pathogen *F. dianthi* Prill. et Del.

In 1942 Bickerton (15) published his studies of the wilt of greenhouse carnation, at Long Island, New York, giving a comprehensive review of the preceding studies. He found that the optimum temperature for wilt is 26.5° C., minimum at 6° and maximum at 36°, and that the main source of infection probably is the pathogen in the soil. His test of ten carnation varieties by inoculation with five isolates of the pathogen showed no wilt in Puritan, Peter Fisher, King Cardinal, Patrician or Main Sunshine; from 60 to 90 percent wilt in Sophelia, Chief Kokomo and Virginia; and 100 percent wilt in My Love and Spectrum Supreme. In 1945 Guba gave a list of carnation varieties resistant to Fusarium wilt. The wilt should not be confused with the foot and stem rot caused by *F. culmorum, F. avenaceum, F. sporotrichoides* and *F. acuminatum* (202). Dowson (43), Wickens (198) and White (196) presented information of diagnostic value in separating Fusarium wilt from other diseases of carnation that may be confused with it.

Verticillium wilt of carnation was reported in 1929 from Germany by Wollenweber (201) who named the fungus *V. cinerescens.* Van Beyma (175) considers it to be a form of *Phialophora* and gives it a new name-combination, *P. cinerescens* (Wr.) van Beyma. In 1935 Wickens (198) gave an account of stem rot and die-back of a perpetual-flowering carnation in England, showing by isolation and inoculation that rot is often a complex of Verticillium wilt caused by *V. cinerescens* Wr., Fusarium wilt caused by *F. dianthi,* stem rot by *F. culmorum* and *F. herbarum,* and die-back apparently by *F. culmorum*. He has not seen any variety widely grown that consistently remained free from the disease, but believes that the search for resistant material, and breeding for resistance, should be persevered with.

In 1936 White (195) published studies of Verticillium wilt of carnation. He agreed with Wickens (198) and added that Verticillium wilt is the most important of the diseases; that there is evidence of a differential susceptibility of varieties towards different strains of the fungus; and that inoculations of tomato, chrysanthemum and aster with this fungus gave negative results. In the test on which these conclusions are based, eight strains of the fungus and two varieties of carnation were employed on a total of 32 plants.

In 1940 White (197) reported the five months of observation of stem-inoculated carnation and other plants exposed to soil infection by *V. cinerescens* showed that *Dianthus caryophylus* was highly resistant, *D. deltoides* immune and *D. caesius* immune from root infection, while *D. chinensis* and *D. barbatus* were very susceptible, and that a large percentage of perpetual-flowering carnation (? *D. chinensis*) grown in contaminated soil also became infected.

Celery

Fusarium yellows of celery was first reported in 1913 by Coons (35) as found at Kalamazoo, Michigan. Later Coons published several brief reports of progress. Further studies of the disease and of the breeding of varieties of celery resistant to it were reported by Coons *et al.* Nelson *et al.* (108) recognized two distinct forms of the yellows, each of wide distribution in all celery districts of Michigan. According to them the first efforts to develop yellows-resistant celery were made in 1916, but owing to the rot in storage all plants selected were lost. In 1919 were selected many apparently yellows-resistant plants from the short strain of the Self-Blanching variety, then most popular with Michigan growers. One of these plants survived soft rot during winter storage and in 1920 produced seed. The seedlings from the latter showed high resistance to the yellows and other characters acceptable to the growers. From them was developed the yellows-resistant short-strain Self-Blanching variety. However, because of the introduction of Golden Plume and the tall strain of Golden Self-Blanching during the period of breeding for resistance in the short strain of Golden Self-Blanching, the resistant short strain never gained widespread use.

In 1921 Coons and Nelson (36), while examining fields of Newark Market green celery which was extensively grown at Kalamazoo and developed some yellows, observed one plant of attractive appearance, with the foliage curled and ruffled, and lighter in color, indicating an improvement in blanching quality over the parent strain. Seedlings of the plant were more uniform and resistant than the regular Newark Market variety. The stock seed was increased in California and distributed to growers for the season of 1925 under the name "Curly Leaf Easy-Blanching".

From 1922 to 1928 the wilt condition in Kalamazoo was not serious, and Golden Plume and the tall strain of Golden Self-Blanching, each possessing some resistance to yellows, were extensively grown. The season of 1929, however, was characterized by drought and high temperatures in July and August, and the yellows developed extensively in plantings of tall Golden Self-Blanching and in some strains of Golden Plume. A number of selections resistant to yellows were made from Golden Self-Blanching celery, but only one plant survived the rot in storage. It yielded some seed, part of which was sown immediately in soil heavily infested with inoculum prepared from the most virulent strains of the two Fusaria causing celery yellows. The seedlings apparently were of uniform type, and extremely high resistance was manifested at all stages of their growth. The new resistant strain was named "Michigan Golden". It gave satisfactory results when tested in Ohio, New York (169) and Colorado. The results of Wilson (199) in Ohio are unusual, showing 6.8 per cent of the yellows. Ryker's tank tests have indicated that Michigan Golden is an immune variety (135).

In comparing the records of the celery wilt on the varieties commonly grown in Kalamazoo in 1915 with the disease as found during 1933 and 1934, Nelson *et al.* (108) state that there is the possibility that prolonged separation of a specific susceptible variety from the infested ground may bring about at least a temporary reduction in the virulence of the pathogen for that variety.

The high yellows resistance in the three celery varieties developed by the Michigan workers does not break down under the high summer temperatures, while the resistance usually found in green varieties of celery readily breaks down, as shown by Ryker (135) and as found by Coons and Nelson (36) in Newark Market green variety in Michigan in 1921.

Chrysanthemum

Verticillium wilt of chrysanthemum was reported from New Jersey in 1923 by Haenseler and in 1925 by Martin; the latter year from Holland by Van der Meer; in 1926 from Michigan by Nelson; in 1929 from Germany by Wollenweber; and in 1932 from Washington by Hubert and Jones who published a detailed account of their studies (64) in 1934. From their observations in greenhouses and on the basis of inoculation tests, Hubert and Jones concluded that the following varieties are resistant to attack by this organism: Adrian's Pride, Ambassador, Antique, Betsy Ross, December Beauty, December Glory, Early Rose, Early Frost, Gold Lode, Golden Bronze, Golden Glory, Golden Queen, Honey Dew, Indianola, Italian Kink, Lustre, Marie D. Peters, Maude Dean, Missoula White, Mrs. Alex Laurie, Mrs. Calkins, Monument, Pink Treasure, and W. H. Waite. The following commonly grown varieties are extremely susceptible to injury: Chadwick, Distinction, Dr. Enguehardt, Favorite, Golden Measure, Justrite, Mrs. Simpson, Seidewitz, and Whittier.

Cotton

In 1892 Atkinson (6) reported cotton wilt caused by a form of *Fusarium* invading vascular tissues of the plant. He described the the fungus as *F. vasinfectum* n. sp. Atkinson found the disease of general occurrence on lighter soils in Alabama and on specimens received from Arkansas. He observed the same fungus causing wilt of okra. At present the disease occurs in all parts of the world where cotton is grown (202).

The fungus causing cotton wilt is variable in cultural characters. No specific difference has been found in differential pathogenicity. It has been noted, however, that different isolates vary in the degree of virulence. In 1927 Neal (103) published an extensive review of cotton wilt and related diseases; and in 1928 Young (204) published his studies of the relation of soil temperature to the development of cotton wilt.

According to Orton (117), the long-staple cotton of the West Indies (*Gossypium barbadense*) was introduced into the United States about 1786 and by the end of the eighteenth century was commonly grown in the southeastern States where long season and abundant rainfall were favorable to it. The cotton grown on the Sea Islands of South Carolina was considered the finest produced in the country, and since the quality would be rapidly lowered by cross pollination with the short-staple upland cottons (*G. hirsutum*), only the long-staple cotton was grown there. Thus in the United States the Sea Islands became the exclusive seed sources of the long-staple cotton which became known as Sea Island variety. The light sandy soils of the Sea Islands, however, are extremely favorable for the development of wilt which by the end of the nineteenth century threatened the culture of Sea Island cotton.

Orton (117) states that the first and best wilt-resistant Sea Island cotton was the Rivers, bred by E. L. Rivers of James Island, South Carolina, in cooperation with the United States Department of Agriculture; that wilt-resistant varieties were developed by other Sea-Island growers; and that because of the use of the resistant cotton at the time of his writing very little loss from wilt occurred in the Sea Islands.

It appears that Orton (111) was the first to breed wilt-resistant upland cottons. For eight years he selected healthy plants in fields where most of the plants were killed by wilt, and tested single-plant progenies under similar field conditions. Thus were developed two new wilt-resistant varieties, Dillon and Dixie. Dillon is of the cluster type, hard to pick, and has never become popular. Dixie proved to be more acceptable to the growers than Dillon and was later used in crosses for further improvement of wilt-resistant cottons.

In 1905 Lewis (89) began his breeding of wilt-resistant cotton varieties in Georgia, by selection and hybridization. From the selections, he developed in 1908 one wilt-resistant variety, Modella. Grant, another wilt-resistant variety, was developed from a new series of selections made in 1909. The first cross made by Lewis, in 1905, was between Dillon and King's Improved; and in 1906 an Egyptian cotton, the Mit-Afifi, which showed nematode resistance he crossed with Orton's wilt-resistant Dixie. In all, 15 susceptible varieties were crossed with Dillon, Dixie, King and Mit-Afifi. Some segregates of the crosses showed more wilt resistance and were much earlier than any other variety available in 1911. Cauthen's (29) tests of Dix-Afifi showed it almost completely free from wilt, while Dillon, Tri-Cook, Dixie, Cook 307–6, Covington-Toole, Modella and Wood, in the order named, showed up as very resistant, ranging from 5.4 to about 15 percent average wilt, as compared with 40 percent in Cook.

Because of boll weevil damage to late varieties of cotton, breeding work of the U. S. Department of Agriculture became directed to the production of earlier and more fruitful wilt-resistant varieties, by the crossing of Dillon and Dixie with the earliest and most fruitful susceptible varieties, Triumph, Cook, Pride of Georgia, Columbia, Coker, Webber, Foster and Trook.

Fahmy (49) started his breeding of wilt-resistant Sakel in Egypt in 1923 by selecting healthy-looking plants of Domain Sakel from seven different fields where the disease was severe. The selections were tested in 1924 in soil inoculated by the fungus in the upper layer, with not much of the disease showing up, even in the non-selected susceptible cotton. In the following years the cotton was planted in large bottomless pots, set out in the field and filled with soil brought from a field where the disease was very severe. A considerable amount of pure culture of the fungus was added to the soil. The selections were tested also in fields with natural heavy infestation. Fahmy used both selfed and open-pollinated seed and always tested the selections in single-plant progenies. He obtained some highly resistant strains of good-quality staple Sakel which he expected shortly to increase for large-scale use. In 1934 Fahmy (50) reported one wilt-immune strain of long-staple cotton, Sakha 4 Gidid, and in 1937 another strain, Giza 27 (51).

Some extensive studies of Fusarium wilt of cotton and breeding for resistance to it have been conducted also in India. In 1939 the Indian Central Cotton Committee (67) reported that of 88 strains of cotton tested, 26 showed a fair degree of resistance to Fusarium wilt at Parbhani, and six out of 11 strains at Latur. It is stated that there is a great demand for the wilt-resistant *Gossypium neglectum verum* 434, a selection from 262, which is hardy, prolific and excellent for spinning. In 1940 Uppal (170) published results of his studies on development of wilt-resistant cotton by hybridization. He states that types of cotton showing 100 percent resistance to wilt have been isolated from the cross B.D.8 x S.7–1 and Broach back-cross (B.D.8 x G.A.26) x B.D.8; that similar results have been obtained with some of the *Gossypium arboreum* var. *neglectum* cottons now being used as parents in crosses with Jarila and N.R.5, with a view to securing a fully resistant type possessing the staple of Jarila and other desirable economic characters for the Khandash tract; and that very promising results were obtained in the Jalgaon section by selections from the Million Dollar and New Million Dollar cottons of Chinese origin. About the same time Uppal, Kulkarni and Ranadive (171) stated that continuous selfing and selection for resistance to Fusarium wilt for three or four years, under conditions of infection approaching the optimum, culminate in the development of types fully resistant to the disease, the homozygous condition being reached more rapidly in some lines than in others; that the attainment of full resistance in B.D.8 crosses, for instance, having been achieved by stages, is assumed to be attributable to the gradual elimination of minor modifying factors; that field selection remains the only practical method of "building up" resistant types as a preliminary to their selection in pot cultures under controlled greenhouse conditions; and that monohybrid segregation for wilt resistance was secured in the F_2 generation of K.F. x 1027 A.L.F., whereas in crosses between Dhulia and Chinese R_1 spotless and New Million Dollar, this character was found to be governed by three complementary factors.

Since the period of the breeding of wilt-resistant cotton by the U. S. Department of Agriculture, a number of new wilt-resistant varieties have been developed by workers in the Cotton Belt. Of these the most recent is a wilt-resistant selection from Empire cotton, reported in 1947 by Smith and Ballard (146). Some private cottonseed companies produced their own resistant varieties, of which 4 in 1 and Coker 100 Wilt of the Coker Pedigreed Seed Company of South Carolina should be mentioned as quite popular at present in the southeastern states.

From 1937 to 1940 a large number of field tests of wilt-resistant and susceptible cotton varieties were conducted by workers in agricultural experiment stations of the Cotton Belt, in cooperation with the Division of Cotton and Other Fiber Crops and Diseases, U. S. Department of Agriculture. The tests showed Seabrook Sea Island cotton to be free from external symptoms of wilt; Cook 307–6, Clevewilt 6, Dixie Triumph 12, Dixie 14–5 and Covington-Toole to be highly resistant, and some other varieties partly so. This seems to indicate that there probably is more than one factor controlling wilt resistance in cotton. Smith (145) reports that one of the varieties tested, Miller 610, showed very little wilt in the absence of root knot but was severely affected by wilt under the conditions of heavy root knot infection.

There is evidence also that resistance to the wilt may be different from resistance to invasion of the plant's vascular system by the fungus. For example, in the reviewer's tests of resistance to wilt of several varieties of cotton, in greenhouse sand cultures inoculated with liquid cultures of the *Fusarium,* Dixie Triumph 12, as in all field tests, showed no external symptoms of wilt; and yet up to 78 percent of the plants were heavily invaded by the fungus high up in the stems.

A dependable technique for determination of relative resistance to wilt is very important. Some contributions to its improvement were made by Armstrong (2) who developed the water-culture method; by Tharp (161) and Sherbakoff who employed sand cultures; and by D. C. Neal who suggests the use of filtrate and extracts of the fungus. Dick (40) made a series of comparative tests using the sand- and water-culture methods without finding either method entirely satisfactory.

The inheritance of wilt resistance was studied by Jenkins *et al.* (69), and resistance was found dominant in the F_1 to about the same degree as the more resistant parent. This is in agreement with Fahmy (50) who found immunity inherited as a simple dominant character. Kulkarni (82) found resistance dominant in the F_1 in some crosses and recessive in others.

Verticillium wilt of cotton was first reported by Carpenter in 1914 as observed on two plants (27), and in 1918 on cotton plants inoculated with *Verticillium* causing okra wilt (28). Shortly afterward Bewley (13, 14) reported successful inoculation of cotton with *Verticillium* from tomato. Even though Carpenter (28) expressed the opinion that some of the reported Fusarium wilt of cotton in the South may actually be Verticillium, there was no further record of the disease until 1929 when it was reported (143) to cause considerable damage to cotton in Tennessee and on the west side of the Mississippi River. In the same report the disease was described as exhibiting symptoms sufficiently different from those of the Fusarium wilt to be readily distinguished in the field. Some workers (134) believe that the symptom differences—defoliation and/or light-green areas on the leaves—are insufficient for separation of the two wilts. The reviewer is convinced, however, that one who becomes familiar with the symptoms will readily recognize Verticillium wilt under ordinary conditions. At present the wilt of cotton has been reported from all continents, and in some cases, as in Peru, it is considered to be the most important disease of cotton (21). There the conditions are especially favorable for Verticillium wilt, and it was reported by Boza (21) that of a large number of varieties of cotton of different species obtained from the United States, Peru and other countries, only Tanguis showed sufficient resistance to the wilt. In 1944 Harland (59) published his studies of Tanguis cotton and of its improvement by a new method. According to him, Tanguis became the standard variety in Peru, except in the Piura zone in the north. The variety was developed by a farmer, Senor Fermin Tanguis, from a single plant of several selected by him for wilt resistance. This variety was characterized by high lint percentage, long staple, high production and greater market value per pound than the variety from which it was selected. According to Harland, Tanguis was discovered in 1908; 8 percent of it was in Peruvian cotton exports in 1918; and in 1933 it constituted 91 percent of the Peruvian crop. On the basis of his study of Tanguis cotton in Trinidad from 1928 onward, Harland came to the conclusion that though the original plant was found in a field of upland cotton, it is neither a chance cross with *G. barbadense* nor a mutant of the upland cotton, as believed by some previous workers, but merely a form of *G. barbadense*. Before Tanguis spread over the country, however, its quality was lowered, and there was evident heterogeneity in the field through mechanical contamination and natural crossing with the other varieties. In 1940 Harland undertook to restore to Tanguis the qualities which it lost and to improve it otherwise, and this was very successfully attained in a relatively short time by systematic year-after-year propagation of best plants from best lines without selfing. The selection was made on the basis of certain standards set for staple length, lint percentage and other characters. Harland gives no data in regard to the Verticillium wilt resistance of the improved Tanguis.

In 1946 Jameson (68) concluded that in future breeding at Uganda for transference of Verticillium resistance to BP50, susceptible but otherwise desirable cotton, B181, resistant, will be used in place of Tanguis which is considered less desirable as a source of resistance; and he reports that the figures for Verticillium resistance of F_1 hybrid between KP28×B181 (resistant) and K40×BP50 (susceptible) are close to geometric means of the two parents, which, he concludes, may be due either to blanding inheritance or to the simple genetic system.

In 1932 Herbert and Hubbart (62) reported *Verticillium* of cotton in California, stating that of the 52 varieties grown on a three-acre test in Joaquin Valley, all except Pima were susceptible to the disease; Pima showed no conspicuous external symptoms of the wilt to the end of the season, though a characteristic vascular discoloration was found in most of the plants at that time. In 1932 Miles (94) reported that all of the upland varieties of cotton resistant to Fusarium wilt which were tested in a *Verticillium*-infested field in Mississippi from 1928 to 1931 were highly susceptible to this wilt. In 1946 Salter (136) reported the release of Verticillium wilt-resistant Acala 1517, in regard to which Leding[1] stated that the breeding work at the U. S. Cotton Field Station, State College, New Mexico, for resistance to Verticillium wilt has been almost entirely through selection. In 1939 the Station changed its production from the California type of Acala cotton to the Acala 1517. It soon was found that the strain was extremely susceptible to Verticillium wilt. Search for resistant or tolerant strains resulted in the development of Acala 1517 WR which possesses considerable disease tolerance and otherwise is a little better than the parent strain. The cotton was released to the growers in 1946, and by 1947 was expected to be grown exclusively in certain counties of New Mexico, Arizona and Texas.

[1] A. R. Leding, in a letter of August, 1947, to C. D. Sherbakoff.

Cowpea

Fusarium wilt of cowpea was first described and its cause, *F. triachiephilum* E. F. Smith, determined by Smith (149) in 1899, and in 1902 Orton (113) published the results of his studies of the disease. It is common in the southeastern States and in parts of California, often being very destructive on light, sandy soil.

In 1900 T. S. Williams, a South Carolina grower, called Orton's attention to the wilt resistance of Iron cowpea. In 1901 this and 16 additional cowpea varieties, besides a number of other legumes, were tested by Orton (113) on Williams' farm in a "cowpea-sick" field. Of all cowpea varieties, Iron was the only one which remained entirely wilt-free to the end of the season. It was also free from root knot. In 1908 Orton (115) reported that among F_1 hybrids between the resistant Iron and some susceptible varieties, some were resistant and some susceptible.

In 1929 Kendrick (77) made 74 selections for resistance to wilt from a field of Black Eye cowpeas in San Joaquin Valley, California. These selections and 25 commercial varieties of cowpeas were tested in 1928. Thirty-two of the selections showed 100 percent wilt; in the others the wilt ranged from 55 to 95 percent. Of these, 30 seemed to possess some resistance. Brabham, Catjang, Columbia, Conch, Early Black, Iron and Virginia Black Eye showed no evidence of wilt.

In 1934 Mackie (92) reported results of the breeding of Black Eye cowpeas for resistance to Fusarium wilt, charcoal rot and nematode root knot. He found only Iron, Victor and Brabham suitable for the purpose. Crosses between Iron and Black Eye gave the best results. In the F_1, resistance to all three diseases was dominant over susceptibility.

Eggplant

Verticillium wilt of eggplant has been known since 1913, at which time the plant was reported by Wollenweber as susceptible when inoculated with the fungus. The disease was soon listed by Orton from Oregon and California. In 1918 Jagar and Stewart reported their study of the disease, and in 1923–26 it was given considerable attention by Haenseler in New Jersey. Additional review of the disease up to 1929 will be found in Rudolph's work

(134). In 1934 Guba (56) published results of his study, reporting that no variety of eggplant has been found which shows any degree of resistance to the disease.

Flax

H. L. Bolley (19) was the first to determine that flax-sick soil, or failure of flax on soil previously used for flax culture, is caused by a species of *Fusarium* which he named *F. lini.*

In 1899 he made the first selections of flax for resistance to the disease, all of which, however, were lost in 1901. In 1902 a mixed lot of seed obtained from a great number of farmers in various parts of the Northwest was planted upon flax-sick soil. A small percentage of plants were resistant to the disease, or only slightly blighted, and matured seed. A part of the seed from 20 of these plants have been given greenhuse culture in comparison with eight samples of common seed flax, all in flax-sick soil. Seed from plants selected for resistance gave very much larger emergence, and of 468 plants that came up only 35 died from any cause; of the 222 check plants 159 were dead from wilt on the same date. Fifty-four days later only two check plants were still alive, while the plants from the progeny of the resistant plants remained strong and in bloom.

Broadfoot (24) found that there are at least eight forms of the fungus which are distinguished by their parasitic action on four varieties of flax and that six of the forms differ from each other in virulence.

According to Tisdale (166), the critical temperature for infection is 15° to 16° C.; later (167) he found that the resistance is only relative, owing to the great modifying effects of environment, especially of temperature. The most resistant strains may wilt at high temperatures, while at low temperatures even susceptible varieties remain unaffected. From certain crosses he found a marked difference in individual plants of the same strain with respect to resistance in the progeny, and concluded that wilt resistance is an inheritable character determined apparently by multiple factors.

Using Wisconsin soil-temperature tanks, steam-sterilized soil, inoculated with pure culture of *F. lini,* Jones and Tisdale (75) found the temperature curve for the disease corresponding closely to that for the growth of the parasite—minimum about 14° C., optimum 24 to 28° C., and maximum 38° C.

In 1923 Barker (8) published his study of wilt resistance in flax, giving a review of the previous studies of the disease. In the studies he used a field that had been cropped continuously to flax since 1914. In addition it was inoculated with pure cultures of the fungus. Annual tests showed it to be heavily infested with the pathogen. Several individual and bulk selections made by Stakman *et al.* (157) were used in the studies. The prevalent belief that wilt resistance can be developed in susceptible varieties by association with the disease, and that the permanence of this resistance is proportional to the length of time the strains have been growing in sick soil, was not supported by the studies. He found that the original selection is the important one; that subsequent selections, unless the plants are heterozygous, are of no avail in changing the resistance of the selections; and that the resistance is not a character acquired by plants only after they are grown for several years on sick soil; that apparently there are different degrees of resistance; that highly-resistant plants can be obtained immediately by selection in the field, and that these will breed true for resistance; that there was no indication that resistance is developed as a result of constant association with the pathogen, but that resistant strains are obtained only by selection and propagation of these genotypes; that some varieties of flax contained no resistant type; that growing plants on sick soil merely eliminates the susceptible strains; that a wilt-resistant strain does not lose its resistance when grown on clean soil, but that under such condition Nature's selection of resistant types ceases to operate; that wilt resistance is only relative; and that the degree of resistance is modified by environment. He reported the characteristics of two superior wilt-resistant varieties, Chippewa and Winona, developed at the Minnesota Station. Winona consistently outyielded most of the other resistant varieties; while Chippewa is moderately resistant also to flax rust caused by *Melampsora lini.* He stated also that there is some evidence of several physiologic races of the pathogen, *F. lini.*

Burnham (25) found that of the strains of flax tested, certain ones were almost completely susceptible, others highly resistant and the remainder intermediate; that the F_1 of resistant by susceptible appears to be intermediate; that the strains of the fungus in the field were not noticeably different in pathogenicity from the single strain of the fungus used in the greenhouse tests; that the wilting in pure resistant strains is not due to segregation; that the fungus was sometimes found in stem tissues of resistant plants which apparently were quite healthy, thus suggesting that the resistance cannot be wholly due to corking off, as suggested by Tisdale (167); that only a small percentage of F_3 families from the cross of a resistant with a susceptible strain were as resistant as the resistant parent, most of the families falling into the highly susceptible and a few into intermediate classes; that the crosses between certain resistant strains of different origin showed a high percentage of wilt, indicating that they may carry different factors for resistance; and that only a small amount of natural crossing occurs in flax. He emphasizes the fact that the great need in the study of wilt resistance is the development of a technique whereby comparable results can be obtained at different times.

Schuster (140) studied the nature of resistance of flax to *Fusarium lini,* using Bison and Punjab varieties of flax, and two physiologic races of the fungus added to sterilized soil before seed was planted. The resistant Bison flax did not exclude *F. lini* but confined the less virulent race of the fungus to the root and crown tissues, and delayed the development and spread of the more virulent race for several days. In the susceptible Punjab variety there was no such limitation for delay. Other results indicated that the fungus must actually be present in the tissues of the stem or branches to cause wilting.

A number of different workers continued Bolley's efforts to develop wilt-resistant flax varieties, and the main results up to 1929 are briefly summarized by Dillman (42). He states that the North Dakota Agricultural Experiment Station has developed four new wilt-resistant varieties of flax—Linota, Buda, Bison and Rio—and that the Minnesota Station has developed wilt-resistant flax varieties—Chippewa, Red Wing and Winona. He gives brief descriptions of all these varieties.

In 1940 Baylis (9) published his studies of flax Fusarium wilt in New Zealand. He concluded that oil varieties of flax, in general, are more susceptible to the wilt than fiber varieties. But the small-seeded North Dakota 52 and 11 and the large-seeded Rio showed marked resistance in a test of 19 varieties on infested soil, with only 0.7, 0.6 and 0.3% infection; the five fiber varieties showed from 1 to 5.6% wilt; and the most susceptible varieties, Punjab and Moose 11/29, displayed 22.8 and 19.3% wilt.

Vanterpool's (177) results of greenhouse experiments with Crown and Royal varieties of flax in naturally flax wilt-infested soil from Ottawa and Saskatoon showed the highly wilt-susceptible flax variety to be less susceptible to wilt when grown in Ottawa than on Saskatoon flax-sick soil. In the case of the former there were 66% dead plants, and in the latter, 97%; whereas a more resistant variety, Royal, was more susceptible to wilt in the Ottawa soil, with 36% dead plants, than in Saskatoon soil, with 12% dead plants.

In 1943 and 1945 Arny (4, 5) published a registration of the Fusarium wilt-resistant flax varieties Biwing, Redson and Arrow. The first two are segregates from the cross Bison by Redwing, made in 1929 at University Farm, St. Paul, Minn., and the third from the cross Bison by Renew, made in 1938. This variety showed immunity from North American races of *Melampsora lini* and the same resistance to Fusarium wilt and *Sphaerella linum* as Bison. Arny (4) reports the following wilt percentages for the varieties mentioned: Bison, 9 and 23; Redwing, 3 and 9; Redson, 3; and Biwing, 9.

In 1945 Milikan (95) reported his studies of flax wilt in Victoria, stating that the Concurrent variety, susceptible to Fusarium wilt and growing in naturally infested soil, may escape the disease when the soil temperature is 55° F. or below. At 75° F. this variety showed 100% wilt, and Bison only 50%. The natural infection was built up by growing a wilt-susceptible variety in the soil. In the test of different varieties in soil with built-up infestation, less than 40% wilt was shown by Argentine 868, Newland × (19 × 112) CI838, Ottawa 770B × Buda C11073, Saginaw × Bombay C1671, and Russian × Argentine CI896. The last-named variety, which is also immune from *Melampsora lini*, has been selected as foundation stock for a future breeding program. This flax, with Concurrent and Liral Crown, in naturally infested soil in the field, in normal winter sowing, showed no wilt 150 days after germination, whereas there was 99.8% wilt in Concurrent and 100% in Liral Crown.

In 1946 Salter (136) reported the development by the Oregon Agricultural Experiment Station, in coöperation with the U. S. Department of Agriculture, of a new fiber-flax variety, Cascade, which is characterized by exceptionally high yield of straw and fiber, good percentage of fiber, immunity from rust, a rating of 4 in resistance to wilt[2], and tall growth habit, but still lacking uniformity, and of somewhat late maturity.

Guayule

Verticillium wilt of guayule (*Parthenium argentatum*) was reported in 1944 by Schnider (137) as occurring in California, Texas and Arizona. He showed also that different strains of the rubber plant vary considerably in their capacity for recovery from infection by *V. albo-atrum.* In 1945 Schnider (138) published his surveys and observations of the disease in California; and the following year Campbell and Presley (26) reported on the importance of the wilt and recommended guayule strain 405 for culture on infested soil.

Mint

Verticillium wilt of peppermint was first reported in 1926 by Nelson (104) who stated that at the time of the report the disease had been under observation and study for two years and that it was causing serious damage on one peppermint farm in Kalamazoo County, Michigan. In 1947 Nelson (105), in a paper on the production of mint-species hybrids resistant to Verticillium wilt, stated that the disease of peppermint and spearmint is very destructive in Michigan, occurs in Indiana and was appearing in Oregon and Washington peppermint fields. He considers peppermint to be an almost completely sterile, complex hybrid, normally blooming from three to four weeks after spearmints. The hybridization work for the development of wilt-resistant peppermint was started in 1942 with a non-commercial spearmint, *Mentha spicata,* found to be highly resistant to wilt and a good pollen producer. It was

[2] This rating was given by E. G. Nelson, Associate Agronomist of Oregon Agr. Exp. Sta., in a letter to the reviewer and was based on tests conducted at Fargo, North Dakota, where the varieties were rated 1 to 10, 1 being entirely susceptible and 10 being extremely resistant.

used as pollen parent, and the commercial variety of peppermint, *Mentha piperita,* as female parent. Since all attempts at hybridization by hand pollination failed, mass insect pollination was resorted to. To make it effective, the blooming period of the spearmint was delayed to that of the peppermint by supplementary illumination of the spearmint field, delayed plantings and other means. Though only a small amount of hybrid seed was obtained, some of the hybrids raised from them mature early and are highly productive of oils of good quality.

Muskmelon

Fusarium wilt of muskmelon was reported by Chupp (32, 33) in 1930 and in 1931 from New York; and in 1933 by Leach (85) from Minnesota. Currence and Leach (37) and Leach and Currence (86) published results of studies of the wilt in Minnesota. In the latter case are presented various aspects and phases of the disease; identification and description of the pathogen, *F. bulbigenum* v. *niveum* f. 2 Leach and Currence; and the results of the breeding of wilt-resistant muskmelons, initiated in 1932. They found also that the *Fusarium* causing wilt of the watermelon does not cause wilt of muskmelon. They found the Persian, Honeydew, Honeyball and Casaba varieties to be relatively resistant but not suitable for culture in Minnesota; the varieties that do well there, *e.g.,* Bender's Surprise, Emerald Gem, Pollock and Sugar Rock, were highly susceptible. A cross of Honeydew by Bender's Surprise was found to be intermediate in resistance and other characters. By continuous selection from selfed and open-pollinized fruits of the cross, some strains of much higher resistance were developed. A large number of *reticulatum* sub-species have been tested without the discovery of significant resistance in any of them. Four of the most promising of the resistant selections thus far developed are described. Stoddard's (159) work in Maryland indicated that the use of low nitrogen-high potassium fertilizers for cantaloups reduced Fusarium wilt damage to the melon crop.

Verticillium wilt of muskmelons was reported in 1918 by van der Lek. Later it was reported by Bewley, Green, Rustrup, Dufrenoy and Rudolph (134). In 1934 Kendrick and Schroeder (78) reported the wilt as occurring in severe form in California.

They found that in greenhouse tests with plants started from seed planted in steam-sterilized soil mixed with cultures of the fungus growing on steamed oats, all of the five main crop-melon varieties grown in California—Casaba, Hale's Best, Honey Ball, Honey Dew and Persian—were susceptible to the disease. However, they observed that in the field where destructive Verticillium wilt occurred, Hale's Best and Honey Dew did not show the disease.

Peas

Peas are subject to two distinctly different, yet typical, vascular diseases caused by *Fusarium*. One of these, known as "wilt", is caused by *F. orthoceras* App. & Wr. var. *pisi* Lindf., and the other, near-wilt, is caused by *F. oxysporum* Schl. f. *8* Snyd. The two wilts are of general occurrence and are destructive on lighter soils from Maryland to California. The near-wilt is prevalent in Europe where it is known as "St. John's wilt".

The wilt develops much faster than near-wilt—in Walker's controlled tests (180) the difference was about three weeks; optimum temperature of wilt is lower; and wilt occurs in the field in circular spots, whereas near-wilt is usually found on scattered plants. Walker (180) noted that all wilt-susceptible pea varieties and with few exceptions all wilt-resistant varieties are susceptible to near-wilt.

Pea wilts well illustrate the importance of recognizing the complexity of the diseases affecting plant roots. The wilt and root-rot complex of peas was reviewed by Snyder and Walker (155); hence, only the more conspicuous phases of it are briefly mentioned here.

Jones (71) in 1923 was the first to contribute to the untangling of the complex situation by his studies of field root rot and stem rot caused by *F. martii* var. *pisi* n. v. In 1925 Jones and Drechsler (72) published their studies of the very important root rot of peas caused by *Aphanomyces euteiches* n. sp.; in 1928 Linford (90) presented his discovery of wilt of peas caused by *Fusarium;* and in 1935 Snyder and Walker (155) published their studies of Fusarium near-wilt of peas.

Linford (90) found sufficient evidence that one species of *Fusarium, F. orthoceras* var. *pisi* n. v., causes pea wilt throughout the known geographic range of the disease, and that its optimum growth in pure culture was at 27°–30° C., minimum at 6.5°–8° C., maximum at 34.5°–35° C. From his inoculatioins of other plants with the pea wilt fungus he found that the fungus invaded *Vicia gigantea* and a variety of *V. faba,* but not cabbage, flax, cowpea, soybeans, beans, lupines and a number of other plants tested. His studies of different cultures gave indication of wide variation in virulence, but no search was made for physiologic specialization.

In regard to wilt resistance in peas, Linford notes that Wisconsin pea growers have recognized for a long time that several varieties of peas, especially Yellow Admiral, Green Admiral and lately Horal, are less subject to disease than the canning peas, though even the resistant varieties sometimes fail. His field observations in 1924 and 1925 showed Green Admiral and Pedigreed Extra Early to be resistant; and in January 1926 he tested wilt resistance of four pea varieties in soil infested with pure culture of the pathogen kept at a temperature favorable for wilt. In the test Horal and Green Admiral remained unaffected by wilt, while most of Alaska and all plants of Horsford died from the disease. On the basis of this and of more extensive greenhouse and field tests, Linford found ten varieties resistant and nine susceptible. His tests of apparently wilt-resistant pea plants selected from susceptible varieties showed that Alaska selections were entirely free from wilt, while the selections from Horsford showed 12 percent wilt; selections from Perfection 50 percent wilt; and the commercial lines ran from 99 to 100 percent wilt. The Alcross, a strain of Alaska developed by E. J. Delwiche, contained 50 percent resistant plants of excellent type, the progenies of the resistant selections remaining entirely wilt resistant. Other resistant selections were of types different from the varieties from which they were selected, corresponding to the plants called "rouges" by the growers. Linford found that the resistance to wilt in peas is nearly complete.

In 1929 Wade (179) published his studies of Fusarium wilt inheritance in canning peas, showing that pea wilt resistance is controlled by a single factor dominant over susceptibility.

Schroeder and Walker (139), using sterile sand and liquid medium inoculated with pure culture of the pathogen, found that by varying the concentration of the medium from 0.1 to 5 times basic concentration at 21° C., the time required for the appearance of complete wilting in susceptible plants varied directly with the concentration of the nutrient solution; in resistant plants disease development was most severe in the 0.1 concentration and nil at the higher concentration. At 27° C. wilt was more severe in the five-times-basic solution, and no difference in symptoms was observed between susceptible and resistant plants. The least amount of wilt in both resistant and susceptible plants occurred in the basic solution; at three-times-basic level the amount of disease was variable; and in the 0.1-basic concentration resistant plants developed a very severe case of slow wilting, while the susceptible plants approached the rapid wilting which takes place in the five-times-basic solution. Their cross inoculations of susceptible and resistant varieties of tomato, pea and cabbage with the corresponding pathogens, under the optimum high temperature and low nutrient sand culture, indicated that the breakdown in pea-wilt resistance was caused only by the pea wilt *Fusarium* and that only the specific pathogens were producing wilt of the other hosts tested.

Snyder and Walker (155) in 1935 published their studies of *Fusarium* near-wilt of peas in which they reviewed the history of studies of pea diseases and presented their studies of the symptoms, etiology and taxonomy of the pathogen of near-wilt, including the St. John's pea disease of Europe which they consider identical with near-wilt in the United States.

Walker and Snyder (186) state that good stocks of varieties of peas like Perfection and Surprise contained few or no wilt-resistant plants and that it was therefore necessary to cross plants of these varieties with resistant plants of other varieties. From the segregates of these crosses were selected lines combining the resistance with the desired type, such as Wisconsin Perfection, Wisconsin Early Sweet, Penin and Merit. The authors state that the resistant varieties are equally resistant to all strains of wilt fungus so far collected, but all are susceptible to root rot and, with a few exceptions, to near-wilt. Since near-wilt develops slowly, it is of importance only on late-planted varieties or on longer-seasoned large-berried sweets. They report some resistance to near-wilt in Horal and Roger's K., and that it is neither as simple nor complete as the resistance to wilt.

In 1944 Walker *et al.* (184) reported finding a major gene for resistance to near-wilt in pea, in a single plant line from a cross of two varieties, Admiral and Pride, neither of which is tolerant to near-wilt. The gene behaves as a dominant to near-wilt susceptibility. The F_3 progenies of a cross of the resistant line with Wisconsin Perfection, resistant to wilt but very susceptible to near-wilt, gave the ratio of 1 resistant: 2 segregating: 1 susceptible. They report that the major genes for wilt and near-wilt are being combined with desirable canning and garden varieties.

Pepper

Verticillium wilt of pepper was reported in 1939 by Snyder and Rudolph (154) who isolated *V. albo-atrum* from diseased pepper plants and reproduced the disease by growing peppers at 70° F. in soil inoculated with culture of the fungus. Of the two varieties tested by them, Red Chili never developed the disease as much as Anaheim Chili.

Potato

Fusarium wilt of potatoes was one of the first wilt diseases described, and was reported in 1904 by Smith and Swingle (150). The disease has been extensively studied but without any attempt at breeding for resistance.

Verticillium wilt of potatoes, reported in 1879 by Reinking and Berthold (128), was the first Verticillium disease described. In 1931 the disease was reviewed by Rudolph (134), with a review of the literature up to 1929 and citation of five reports of variation in resistance and susceptibility of different potato varieties. In 1946 Yarwood (203) reported that premature dying of Netted Gem potatoes in San Juan district, San Benito, California, is associated with heavy infection with *Verticillium albo-atrum* and *Corticium vagum,* with a few larger and much longer-lived plants closely resembling "giant hills", commonly thought to be due to a virus, being found in the fields. These plants Yarwood found to be resistant to *Verticillium albo-atrum* and giving 63% greater yield than the plants dying earlier. All progenies of these later-maturing plants varied in maturity and in most other characters. Tests in 1942–45 showed these potatoes to be also less susceptible to late and early blight, to *C. vagum* and to frost injury.

Raspberry and Other Brambles

Verticillium wilt of raspberries was first reported in 1912 by Lawrence; in 1925 Harris described it on several English red-raspberry varieties, *Rubus idaeus;* in 1926 Berkeley and Jackson described the disease on American red raspberry varieties, *R. strigosus;* in 1927 Rudolph reported Verticillium wilt resistance of the red and black Loganberry; in 1931 Rudolph (134) reviewed the literature up to 1929; and in 1936 Zeller (205) published his studies of the disease in Oregon where it has been especially destructive on black raspberries. Zeller made tests of the susceptibility of different brambles. For inoculation of the plants, he placed in the soil at planting time ample culture of the *Verticillium* growing on sweet clover stems, so that the roots of the tip plant were in contact with the fungus. He found that (*a*) all varieties of black and purple raspberries are susceptible to the disease, though some may live for several years after inoculation; (*b*) the Cuthbert red raspberry is very resistant, whereas Ranere, Chief, Herbert, Red Autwerp, Latham and Sunbeam show considerable infection, and Lloyd George is susceptible to the Oregon strain of *Verticillium;* (*c*) the Evergreen, Himalaya, Lawton and Wild Northwestern Trailing blackberries, *R. macropetalus,* showed high resistance, while some infection was noted in the Cory Thornless, Kittatinny, Logan, Lucretia, Mammouth, Phenomenal, Snyder and Stuart blackberries; some Asiatic varieties showed very high resistance and others high tolerance to *Verticillium.*

Sann Hemp

Fusarium wilt of Sann hemp (*Crotalaria juncea*) is a disease of importance in India and in some other places. Breeding in India for resistance was reported in 1937 by Uppal (171) who stated that the seed of the homozygous strain D-IX of Sann hemp, resistant to *Fusarium* wilt, is multiplied during the year.

Spinach

Fusarium wilt of spinach was first described in 1919 by Hungerford who considered it to be caused by *F. spinaciae* Sher. In 1926 Taubenhaus reported a similar disease from Texas; and in 1938 Cook and Nugent stated that the disease was found by them in

Virginia in 1930. In 1947 Cook, Nugent, Paris and Porter (34) published their studies of the disease. They reported development of the resistant strain of Virginia Savoy spinach by repeated selection of resistant plants in wilt-infested soil and in soil inoculated by the sowing of oats on which the fungus had been cultured and then planting the spinach seed in the same rows, with precautions against cross pollination of the plants selected for resistance with those of common Savoy spinach. The selection work was initiated in 1936, and in 1942 the resistant strain obtained was tested in commercial plantings. The tests were made in fields in which spinach had been severely damaged by the wilt. They showed excellent resistance to the disease and made a yield 60 percent higher than that of the regular commercial variety. Since then the resistance has been maintained and improved; and in 1946 seed from the last-tested lot was released to seedmen for multiplication.

Strawberry

Verticillium albo-atrum was reported as a definitely proved cause of strawberry wilt in 1931 by Thomas (162). He published his studies the next year (163) and showed the wilt to be of considerable importance in California on land previously cropped to tomatoes under conditions favorable for Verticillium wilt. He reported also a conspicuous difference in the susceptibility of different varieties to the disease, the Marshal type (Banner, Oregon, Oregon Plum), Klondike and Blakemore appearing to be comparatively resistant, Nick Ohmar and Capitolia very susceptible.

Sweetpotato

Stem rot, or wilt, of sweetpotato was first described by Halsted (58) from New Jersey in 1890. Since then it has been reported by several other workers, but without any definite study of the cause, until 1914 when Harter and Field (61) published their work. They found that it was caused by a Fusarium, *F. batatis* Wr., isolated by them from discolored vascular tissues of diseased sweetpotato plants. Later they found that another related species, *F. hyperoxysporum* Wr., isolated from similar diseased material, also is pathogenic on sweetpotatoes and produces similar wilt symptoms. *F. hyperoxysporum* was the only pathogen isolated by them from sweetpotato stem rot specimens received from California, and in general it was the more frequently encountered pathogen of the two. *F. batatis* can be readily distinguished on a morphologic basis from the other pathogen and other wilt-causing Fusaria.

Poole (118) found White Yam and Red Brazil entirely free from wilt; Triumph practically free; Dooley and Jersey Strain 116 not seriously affected; Jersey Strain 105, Porto Rico, Nancy Hall, and Jersey selections 104, 108, 102 and 103 showing from 50 to 85 percent wilt, in the order mentioned.

Breeding sweetpotatoes by selection from seedlings and by hybridization was initiated in Louisiana by Miller *et al.* (96, 97) who during 1939 and 1938 devised a method of growing sweetpotatoes that enables them to bloom and set seed. Of the several methods tried, they succeeded best by carrying sweetpotato plants through the winter in ten-inch pots in the greenhouse; shifting these into the field in the spring after danger of frost passes; training the vines in the field to tall wire trellises; and, when the vines are well grown, by girdling the vines about six inches from the ground. They found that seed production varies considerably in different varieties and that some set seed better in the fall and some in the spring.

Miller *et al.* (98, 99) reported the results of their breeding of sweetpotatoes for uniform shape, high yield and disease resistance, including resistance to stem rot. One of the seedlings, L4-5, selected for high yield and high starch content, has been found by pathologists to be uniformly free from stem rot. In 1944 Harter (60) stated that none of the varieties so far tested is entirely immune from wilt, but that White Yam, Southern Queen, Triumph, Red Brazil, Yellow Strasburg, Key West and Dohomey can be grown with complete safety in infested soil; and that Yellow Jersey, Big Stem Jersey, Gold Skin, Nancy Hall, Porto Rico, Red Jersey, Georgia, Nancy Gold, Kansas 40 and Maryland Golden are highly susceptible to stem rot. At present there are several very promising stem rot-resistant or -tolerant sweetpotato varieties and crosses, developed mostly by workers at the Louisiana Agricultural Experiment Station and in the U. S. Department of Agriculture.

Tobacco

Fusarium wilt of tobacco, caused by *F. oxysporum* Schl. v. *nicotianae* Johnson, is found in most of the northeastern tobacco-growing States, in Canada, Ceylon and Indo-China, and in the warmer parts of France and Russia (202).

In 1934 Valleau (173) reported results from the breeding of wilt-resistant white burley tobacco. He found that 1,025 F_2 hybrids between resistant Turkish and susceptible white burley segregated into 272 resistant and 753 susceptible when inoculated with the pathogen at setting time, indicating that the resistance is a simple Mendelian recessive and that the varieties Pennsylvania Havana 18, Turkish and Baur's low-nicotine Havana and Cuba are practically immune from Fusarium wilt. Later Valleau *et al.* (174) reported that dark varieties of tobacco in Kentucky were 50 percent resistant to wilt when inoculated at setting time; that commonly-grown varieties of burley are highly susceptible; and that Kentucky 33, developed by them, is highly resistant to wilt and to black root rot and is being grown by many farmers to whom the wilt is of importance. Breeding work at the Kentucky Agricultural Experiment Station has been continued and has resulted in the development of a new variety, K34 (79), resistant to black root rot, Fusarium wilt and mosaic; and in further improvement of burley tobaccos by selection from crosses between Ky. 33, Ky. 34 and Barnett. Some F_3 segregates of the crosses Barnett with the two Kentucky varieties showed a high degree of resistance to Fusarium wilt and excellent plant characters (80).

Tomato

Fusarium wilt of tomato and the pathogen (*F. lycopersici* Brushi) have been intensively studied since the early years of this century. An extensive review of the studies dealing with the disease and of the work on breeding for wilt resistance was made by Bohn and Tucker (17).

Essary (47) in Tennessee and Edgerton and Moreland (44) in Louisiana started the breeding of wilt-resistant tomatoes in the same year, 1910, and Norton (109) began similar work in Maryland in 1912. Essary developed several wilt-resistant lines by selection of healthy plants in six fields. Toward the end of the season only a few plants were left unaffected or only slightly affected. One of these resistant lines, named "Tennessee Pink", was selected from a field of the Beauty (48) and another, Tennessee

Red, without mention of its origin. Essary tested wilt resistance primarily by growing the plants in soil naturally heavily infested with the pathogen.

Edgerton and Moreland (45) reported selection of apparently wilt-resistant plants from the Acme tomato in 1910 in a field where only a small number of plants survived from wilt. The progeny of only one of the selections showed resistance to wilt the following year. The strain was named "Louisiana Wilt Resistant". The tomato, however, was late maturing and a light yielder. Hence, in 1912 crosses were made between it and Langdon's Earliana, from which were developed two wilt-resistant varieties, the Louisiana Red and Louisiana Pink, both highly resistant to wilt and better yielders under Louisiana conditions than the other wilt-resistant tomatoes available at the time. Edgerton and Moreland, in all their tests, used both infested fields and pots of infested soil in the greenhouse. This combination of methods has since become standard practice with nearly all breeders interested in developing wilt resistance of tomatoes and other crops.

Norton 109) started his tomato tests for wilt resistance in 1912 in a naturally heavily infested field. He worked with a number of tomato varieties, including five wilt-resistant lines developed by Essary. Of the many selections made in 1912, only one gave high wilt-resistant progeny in the following season. In the winter of 1912–13 several of the varieties, which in his test showed most resistance to disease, were crossed with the best susceptible tomatoes. None of the F_1 plants, however, showed any marked wilt resistance.

In 1922 Pritchard (126) reported development of four wilt-resistant tomato varieties: Arlington, Columbia, Marvel and Norton. The first two varieties are very much alike and were selected from the same variety, the Greater Baltimore; Marvel was selected from a French variety, Marveille des Marchés; and Norton was obtained from a selection made by Norton.

Most of the wilt-resistant varieties developed by direct selection from susceptible varieties were inferior to the best commercial varieties. The more popular wilt-resistant varieties were obtained from crosses between wilt-resistant and high-quality susceptible varieties: Marglobe, developed by Pritchard from a cross between Marvel and Globe, is the most generally grown variety of tomato in the States where wilt is of greatest importance; Break O'Day, another popular variety developed by Pritchard and Porte (127), was obtained from a cross between Marglobe and Marvana, the latter from a cross of Marvel on Earliana; Konora was developed by White (194) in cooperation with other Kansas workers, from Norton by John Baer; Huelsen and Gills (65) developed two wilt-resistant greenhouse tomatoes from a cross of Louisiana Pink by Grand Rapids Forcing; Rutgers, released by New Jersey Agricultural Experiment Station[8], was developed from a cross of Marglobe by J. T. P., made in 1928 by Campbell Soup Company. Prior to Bohn and Tucker's (17) report of the finding in 1939 of a wilt-immune tomato, the resistance employed in the breeding was that which enabled the resistant plants to live sufficiently long to produce a nearly normal crop of fruit only under relatively mild wilt conditions. However, when the conditions were very favorable for the disease the resistant tomatoes were also severely affected by it. Resistance of a very different nature, not affected by the disease even under conditions of heaviest infestation and with temperature and moisture most favorable to the disease, was found by Bohn and Tucker (17) and by Porte and Wellman (121) in the same strain of Red Currant tomato (*Lycopersicon pimpinellifolium*) brought from South America by the U. S. Division of Plant Exploration and Introduction about 1930. This resistance, considered as immunity by Bohn and Tucker and as complete resistance by Porte and Wellman, was found by both teams of workers to be controlled by a single dominant factor. Neither team observed any visibly affected plant in the Red Currant line or in F_1 plants of a cross between it and a susceptible variety. Bohn and Tucker (7) found that progenies of the first back-crosses of the F_1 plants to commercial varieties yielded proportions of immune and susceptible plants corresponding to those expected when the single dominant factor is involved.

With the wilt resistance of the Bohn and Tucker's immune lines and of the Pan America (120), the breeder now is in position to incorporate the high resistance into any horticultural variety of tomato without much difficulty. Using Bohn and Tucker's (17) wilt-resistant tomatoes by crossing with and back-crossing to Globe, Alexander (1) reported in 1947 the development of a greenhouse tomato resistant strain 1 of *Fusarium* causing tomato wilt, stating, however, that Pan America is susceptible to strain 2 of *Fusarium* recently found by him.

---

[8] 56th Ann. Rept. N. J. Agr. Exp. Sta.: 51 and 52. 1935.

Verticillium wilt of tomatoes, according to Rudolph (134), was first definitely reported in 1918 from the United States by Jagger and Stewart and from Holland by van der Lek; and since then the disease has been found widespread in the United States and Canada, and throughout Europe wherever tomatoes are grown. Rudolph (134) made an extensive review of the disease up to 1929. In 1937 Lesley and Shapovalov (88) announced the development in California of a variety named "Riverside" which was resistant to Fusarium and Verticillium wilts. The variety originated from a cross made in 1928 between Cal 2, a Verticillium wilt-resistant strain of the commercial variety Santa Clara, and Fusarium wilt-resistant Marvana, by the ordinary process of selection for eight years. In 1939 Shapovalov and Rudolph (141) announced the development of a new Verticillium wilt-resistant tomato, Essar, for canning in California. Essar was developed from an apparently chance hybrid which occurred in the writer's test plots, comprised of many commercial varieties. Several lines from the selection were tested for Verticillium-wilt resistance on ground heavily infested with the fungus. Finally in the eighth generation and after two additional years of testing, the best line was distributed in 1939 under the name Essar. In 1940 Shapovalov and Lesley (142) published in some detail their study of the resistance of Riverside tomato variety to Fusarium and Verticillium wilts. In 1946 results of the testing in Utah of Verticillium wilt-resistance of 17 tomato species and 60 hybrid selections were reported (129). The tests showed high resistance in Peruvian wild tomato and its hybrids with common tomato, none of which is yet acceptable commercially.

Watermelon

Fusarium wilt of watermelon was first described by Smith (149) in 1899 as occurring in South Carolina. He named the pathogen *F. niveum.*

W. A. Orton in 1902 (112) and 1911 (116) published the results of his breeding of wilt-resistant watermelons. He found that all edible varieties of watermelon are very susceptible to the disease, but that the stock citron melon is resistant to it and, when crossed with an edible variety, Eden, gives some wilt-resistant segregates in F_2 and further generations. F_1 was susceptible and had to be grown in soil free from contamination with the fungus. From a backcross of F_1 to Eden, Orton finally developed his wilt-resistant edible watermelon Conqueror.

Since Smith and Orton's first reports, the disease has been found to be of very general occurrence in the United States wherever watermelons are extensively grown, and also in South Australia, Southern Europe and Japan (202).

The Conqueror had not found favor with the growers and was almost lost. In 1925 interest in the breeding of wilt-resistant watermelons was revived by Layton, Melhus, Porter and Wilson of the Iowa Agricultural Experiment Station. The studies of these workers and others are comprehensively reviewed by Wilson (199) and Porter (122).

The Iowa workers developed Iowa Belle, a chance cross of Conqueror with an unknown variety, Iowa King, of the same origin as Iowa Belle; Pride of Muscatine, from Kleckley Sweet, first reported by Layton and Wilson (84); and another wilt-resistant variety, from Kleckley Sweet, named "Improved Kleckley Sweet No. 6". They also found that a Japanese variety, recorded as Japan 7, was highly resistant to wilt. A cross of it by Stone Mountain gave them another wilt-resistant variety, Improved Stone Mountain No. 5. In Australia Wenholz (193) reported the development of wilt-resistant variety Hawksbury, a black-seeded selection from Grey Monarch. Walker (189) reported the development in Florida of wilt-resistant variety Leesburg by selection from Kleckley Sweet.

Wilson (200) carried out extensive studies of the host and parasite relationship in varieties and strains of watermelon resistant to *F. niveum*. He found that infection of seedlings at or shortly after germination of the seed, combined with favorable conditions for the pathogen, leads to rapid invasion of the xylem vessels with mycelium in the primary root, resulting in a high percentage of wilting. Older plants apparently succumb from a series of internal pathological disturbances caused by the fungus growth in them, involving the accumulation of gum-like materials, tyloses and mycelium in the xylem vessels. In the older resistant plants which survived in fields heavily infested with the *Fusarium,* he found bands of gum-like material surrounding the older xylem near the center of the root axis, while the secondary xylem remained unaffected. No xylem free from gum-like material was observed in infected disease-susceptible plants, but no gum or tyloses of appreciable quantity was found in resistant and susceptible plants grown free from the pathogen.

Wilson (200) states that the greenhouse indexing of resistant seedlings in steam-sterilized soil inoculated with the pathogen has proved of value in the testing of Iowa Belle and Pride of Muscatine varieties, but Iowa King selections failed to show a significant difference when compared with susceptible checks. In field studies on soils heavily infested with the wilt pathogen, resistant plants seemed more resistant with age, the heaviest mortality being within the first 16 to 24 days, after which a few plants wilted, while susceptible checks continued to wilt throughout the season. Irrespective of the ordinary changes in air and soil temperatures and precipitation there was an upward trend in the percentage average daily wilt which reached a maximum at 23 to 39 days after the planting of susceptible seedlings and at 16 to 24 days in the case of resistant seedlings. Iowa Belle appeared to be especially desirable as a resistant parent.

Porter (122) reviewed extensively the main features of watermelon breeding for wilt resistance in general, offered suggestions for future studies and reported the development in California of a high-quality wilt-resistant Klondike R7, a green-skinned melon answering the local demand for a wilt-resistant variety of Klondike type. Another wilt-resistant, striped melon, Blue Ribbon, was developed in California by Porter and Jones (124) from a cross between Klondike 11, susceptible, and Klondike 7, resistant.

The mode of inheritance of resistance to wilt in watermelons has been studied by a number of workers. Orton and the Iowa Agricultural Experiment Station workers considered the resistance to be a recessive character. Bennett (11), using F_3 families from a cross of resistant Russian with susceptible Early Fordhook, came to the conclusion that resistance probably is dependent on several factors and that the reaction of F_1 is intermediate between that of the parents. Porter (123) found Chinese to be resistant, citron with 18% wilt and the African Forage immune. The F_1 of the crosses of these resistant with susceptible watermelons were as susceptible as the susceptible parent. Resistance in F_2 varied with the resistance of the resistant parent. Welch and Melhus (191), in their studies of wilt resistance in F_1 hybrid watermelons, found in 1937 that Dixie Queen crossed with wilt-resistant inbred selections from Iowa Belle x (Iowa Belle x Jugoslavia 7) gave 70 to 85 percent wilt-resistant plants. A test of the F_1 in 1940 gave 71.2 percent resistant plants. In another experiment F_1 seed from 12 additional crosses between wilt-resistant and wilt-susceptible lines were tested in a field heavily infested with the fungus. F_1 of two of the crosses, Japan 7 by Thornmound Grey and Japan 7 by Dixie Queen, were 80 percent wilt resistant. In the other crosses F_1 resistance was from 50 percent to zero. Dixie Queen hills were all killed by wilt. The authors conclude that while Orton and Porter and Melhus found resistance to be recessive, the present studies show the inheritance of crosses between watermelon and citron is different from those involving only watermelon varieties; the F_1 between susceptible edible and resistant edible may or may not be susceptible. The highest resistance in F_1 of the crosses studied was found to be around 75 percent.

Of the more recently developed Fusarium wilt-resistant watermelons may be mentioned Walker's (52) Blacklee variety in 1941; Georgia Wilt Resistant (54) in 1943; Georgia No. 2 (55) in 1946; and Tennessee and Mississippi (46) cooperative development of Miles wilt-resistant watermelon in 1947.

BIBLIOGRAPHY

1. ALEXANDER, L. J. Development of Fusarium-wilt-resistant glasshouse tomato variety. Phytopath. 37: 1. 1947 [Abstract].
2. ARMSTRONG, G. M., J. D. MCLACHLAN and R. WEINDLING. A solution culture infection method used in the study of Fusarium wilt. Phytopath. 31: 549–553. 1941.
3. ————, B. S. HAWKINS, and C. C. BENNETT. Cross inoculations with isolates of Fusaria from cotton, tobacco, and certain other plants subject to wilt. Phytopath. 32: 685–689. 1942.
4. ARNY, A. C. Flax varieties registered, I. Jour. Am. Soc. Agron. 35: 823–828. 1943.
5. ————. Registration of improved flax varieties, III. Jour. Am. Soc. Agron. 37: 646–648. 1945.
6. ATKINSON, GEO. F. Some diseases of cotton. Ala. Agr. Exp. Sta., Bul. 41: 19–29. 1892.

7. Baker, K. F., W. C. Snyder and H. N. Hansen. Some hosts of Verticillium in California. Pl. Dis. Rep. **24**, **20**: 424–425. 1940.
8. Barker, H. D. Studies of wilt resistance in flax. Minn. Agr. Exp. Sta., Tech. Bul. 20: 1–42. 1923.
9. Baylis, S. T. S. Flax wilt (*Fusarium lini*) in New Zealand. N. Z. Jour. Sci. Tech. **22**, 3: 157–162. 1940. [Rev. App. Myc. **20**: 408. 1941].
10. Beach, Walter S. The Fusarium wilt of china aster. 20th Mich. Acad. Sci. Rep., pp. 282–307. 1918.
11. Bennett, L. S. Studies on the inheritance of resistance to wilt (*Fusarium niveum*) in watermelon. Jour. Agr. Res. **53**: 295–306. 1936.
12. Berkely, J. H., G. O. Madden and R. S. Wilson. Verticillium wilts in Ontario. Sci. Agr. **11**: 739–759. July, 1931.
13. Bewley, W. F. "Sleepy disease" or wilt of the tomato. Sixth Ann. Rep. (1920) Cheshunt Exp & Res. Sta. 26–38. 1921.
14. ————. "Sleepy disease" of the tomato. Ann. App. Biol. **9**: 116–134. 1922.
15. Bickerton, J. M. Fusarium wilt of carnations caused by *Fusarium dianthi* Prill. et Del. Cornell Agr. Exp. Sta., Bul. 788: 31. 1942.
16. Blank, L. M. Uniformity in pathogenicity and cultural behavior among strains of the cabbage-yellows organism. Jour. Agr. Res. **48**: 401–409 1934.
17. Bohn, G. W. and C. M. Tucker. Immunity to Fusarium wilt in tomato. Science **89**: 603–604. 1939.
18. ————. Studies on Fusarium wilt of the tomato. I. Immunity in *Lycopersicon pimpinellifolium* Mill. and its inheritance in hybrids. Mo. Agr. Exp. Sta., Res. Bul. 311: 3–82. 1940.
19. Bolley, H. L. A preliminary note on the cause of flax-sick soil. *Fusarium lini* sp. nov. Proc. Soc. Prom. Agr. Sci., pp. 42–46. 1901.
20. —— ——. Flax wilt and flax sick soil. No. Dak. Agr. Exp. Sta., Bul. 50: 27–57. 1901.
21. Boza, Teodoro B. Memoria de la Estacion Exper. Agricola de la Molina. Lima, Peru 294–312. 1938.
22. Brandes, E. W. Distribution of *Fusarium cubense* E. F. S., the cause of banana wilt. Rep. Mich. Acad. Sci. **20**: 271–275. 1918.
23. ————. Report of the Plant Pathologist. Ann. Rep. Porto Rico Agr. Exp. Sta. **1916**: 29–31.
24. Broadfoot, W. C. Physiological specialization of *Fusarium lini* Bolley. Phytopath. **16**: 951–978. 1926.
25. Burnham, C. R. The inheritance of Fusarium wilt resistance in flax. Jour. Am. Soc. Agr. **24**: 734–754. 1932.
26. Campbell, W. A. and J. T. Presley. Diseases of guayule and their control. U. S. Dept. Agr., Cir. 749: 1–42. May, 1946.
27. Carpenter, C. W. The Verticillium problem. Phytopath. **4**: 393. 1914.
28. ————. Wilt disease of okra and the Verticillium problem. Jour. Agr. Res. **12**: 529–546. 1918.
29. Cauthen, E. F. Wilt-resistant varieties of cotton. Ala. Agr. Exp. Sta., Bul. 189: 67–88 1916.
30. Cheema, G. S. and S. S. Bhat. Banana breeding of the Imperial College of Tropical Agriculture, Trinidad. Ind. Jour. Agr. Sci. **6**: 484–501. 1936.
31. Cheesman, E. E. Principles of banana breeding. Trop. Agr. **11**: 132–137, 176–181, 203–209. June–Aug., 1934.
32. Chupp, Charles. Fusarium wilt of muskmelons. Pl. Dis. Rep. **14**: 160. 1930.

33. ———. Fusarium on muskmelons. Pl. Dis. Rep. Supp. **81**: 116. 1931.
34. Cook, H. T., T. J. Nugent, G. K. Paris and R. P. Porter. Fusarium wilt of spinach and the development of wilt resistant variety. Va. Truck Exp. Sta., Bul. 110: 1810–1820. 1947.
35. Coons, G. H. The Michigan plant disease survey for 1914. Celery diseases. Mich. Acad. Sci. Rep. 17: 126–128. 1915.
36. ——— and R. Nelson. Celery yellows. Phytopath. **11**: 54. 1921. [Abstract].
37. Currence, T. M. and J. G. Leach. Progress in developing muskmelon strains resistant to Fusarium. Proc. Am. Soc. Hort. Sci. **32**: 481–482. 1934.
38. Darrow, G. M. and G. F. Waldo. Strawberry culture: Western United States. U. S. Dept. Agr., Farm. Bul. 1027: 26. 1941.
39. Delacroix, G. Sur la maladie des ocillets produite par le *Fusarium Dianthi* (Prill. et Delac.). Acad. Sci. (Paris) Compt. Rend. **131**: 961–963. 1900.
40. Dick, J. B. Methods for greenhouse determination of resistance to cotton wilt of varieties and strains of cotton. (Mimeographed). A thesis. Grad. School Ala. Polytech. Inst. 39. 1944.
41. Dickson, J. G. Influence of soil temperature and moisture on the development of the seedling-blight of wheat and corn caused by *Gibberella saubinetii.* Jour. Agr. Res. **23**: 837–870. 1923.
42. Dillman, A. C. Flax resistant to wilt developed at Experiment Stations. U. S. Dept. Agr., Yearbook **1928**: 296–297. 1929.
43. Dowson, W. J. On the stem rot or wilt disease of carnations. Ann. App. Biol. 16: 261–280. 1929.
44. Edgerton, C. W. and C. C. Moreland. Diseases of the tomato in Louisiana. La. Agr. Exp. Sta., Bul. 142. 1913.
45. ——— and ———. Tests of the wilt resistance of different tomato varieties. La. Agr. Exp. Sta., Bul. 184: 1–24. 1921.
46. Epps, James M. A new wilt-resistant watermelon. Phytopath. **37**: 433. 1947. [Abstract].
47. Essary, S. H. Notes on tomato diseases with results of selection for resistance. Tenn. Agr. Exp. Sta., Bul. 95. 1912.
48. ———. Some common tomato diseases. Tenn. Agr. Exp. Sta., Circ. 31: 4. 1934.
49. Fahmy, Tewfik. The Fusarium disease of cotton (wilt) and its control. Min. Agr., Egypt. Leaflet No. 11: 1–16. 1929.
50. ———. The selection of wilt immune strains of long staple cotton (Sakha 4 Gidid). Min. Agr. Egypt, Tech. & Sci. Serv. (Myc. Sect.)., Bul. 130. 1934.
51. ———. Giza 27, a wilt immune strain of long staple cotton. Egypt Agr. Min. Bul. 176: 1–13. 1937.
52. Fla. Agr. Exp. Sta., Ann. Rep. **1940/41**: 104, 105. 1941.
53. Galloway, B. T. Am. Gard. 17: 518. 1896.
54. Ga. Agr. Exp. Sta., 55th Ann. Rep. 44. 1942–43.
55. ———. 58th Ann. Rep. 73. 1945–46.
56. Guba, E. F. Control of eggplant Verticillium. Phytopath. **24**: 906–15. Aug., 1934.
57. ———. Carnation wilt diseases and their control. Mass. Agr. Exp. Sta., Bul. 427: 64. 1945.
58. Halsted, B. D. Some fungous diseases of the sweet potato. N. J. Agr. Exp. Sta., Bul. 76: 33. 1890.
59. Harland, S. C. The selection experiment with Peruvian Tanguis cotton. Sociedad Nacional Agraria Institute of Cotton Genetics, 1943. Lima, Peru, Bul. 1: 3–98. Bib. 1944.

60. Harter, L. L. Sweet potato diseases. U. S. Dept. Agr., Farm. Bul. 1059: 26. 1944.

61. ———— and Ethel C. Field. The stem rot of the sweet potato. Phytopath. 4: 279–303. 1914.

62. Herbert, F. W. and J. W. Hubbard. Verticillium wilt (hadromycosis) of cotton in the San Joaquin Valley of California. U. S. Dept. Agr., Circ. 211: 7. 1932.

63. Higging, J. E. The banana in Hawaii. Hawaii Agr. Exp. Sta., Bul. 7: 53. 1904.

64. Hubert, G. A. and L. K. Jones. The Verticillium disease of chrysanthemum. Washington Agr. Exp. Sta. Bul. 290: 14. 1934.

65. Huelsen, W. A. and M. C. Gillis. Breeding two new varieties of greenhouse tomatoes resistant to Fusarium wilt. Ill. Agr. Exp. Sta., Bul. 361: 408–434. 1930.

66. Jackson, A. B. The Fusarium wilt of china asters. Sci. Agr. 7: 223–247. 1927.

67. Indian Central Cotton Committee, Ann. Rep. for the year ending 31st Aug., 1938. 1939. [Rev. App. Myc. 19: 15. 1940].

68. Jameson, J. D. Annual Report of the Department of Agriculture, Uganda Protectorate, for the period 1st July, 1944–30th June, 1945, Part II: 32–33. 1946.

69. Jenkins, W. H., E. E. Hall and J. O. Ware. 52nd Ann. Rep. S. C. Agr. Exp. Sta. 1939.

70. Johnson, James. Fusarium wilt of tobacco. Jour. Agr. Res. 20: 515–535. 1921.

71. Jones, Fred Reuel. Stem and rootrot of peas in the United States caused by species of Fusarium. Jour. Agr. Res. 26: 459–475. 1923.

72. ———— and C. Drechsler. Rootrot of peas in the United States caused by *Aphanomyces euteiches* (n. sp.). Jour. Agr. Res. 30: 293–325. 1925.

73. Jones, L. R. and J. C. Gilman. The control of cabbage yellows through disease resistance. Wis. Agr. Exp. Sta., Res. Bul. 38: 70. 1915.

74. ———— and Regina S. Riker. Wisconsin studies on aster diseases and their control. Wis. Agr. Exp. Sta., Res. Bul. 111: 39. 1931.

75. ———— and W. B. Tisdale. The influence of soil temperature upon the development of flax wilt. Phytopath. 12: 409–413. 1922.

76. ————, J. C. Walker and W. B. Tisdale. Fusarium-resistant cabbage. Wis. Agr. Exp. Sta. Res., Bul. 48: 3–34. 1920.

77. Kendrick, J. B. Susceptibility of cowpea varieties and selections to Fusarium wilt and root knot. Phytopath. 19: 1145. 1929. [Abstract].

78. ———— and F. R. Schroeder. Inoculation tests with Verticillium wilt of muskmelons. Phytopath. 24: 1250–1252. 1934.

79. Ky. Agr. Exp. Sta., 57th Ann. Rep. 24–26. 1944.

80. ————. 58th Ann. Rep. 41, 42. 1945.

81. Klebahn, H. Beitrage zur Kenntnis der Fungi Imperfecti. I. Eine Verticillium-krankheit auf Dahlien. Myc. Centralbl. 3: 49–66. 1913.

82. Kulkurni, G. S. Studies in wilt disease of cotton in the Bombay Presidency. India Jour. Agr. Sci. 4: 976–1048. 1934.

83. Kuprianoff, V. A. and M. V. Gorlenko. Parasites of tobacco in the region of Dryazkin Research Station observed in 1929. Morbi Plantarum, Leningrad, 19: 182–192. 1930. [Russian].

84. Layton, D. V. and J. J. Wilson. Three new wilt-resistant varieties of watermelons. Phytopath. 21: 114. 1931. [Abstract].

85. Leach, J. G. A destructive Fusarium wilt of muskmelons. Phytopath. 23: 554–556. 1933.

86. ———— and T. M. Currence. Fusarium wilt of muskmelons in Minnesota. Minn. Agr. Exp. Sta., Tech. Bul. 129: 3–32. 1938.

87. Leach, R. Mycological work. Rep. Dept. Agr. Jamaica, 1942–43. 1944. [Rev. App. Myc. 23: 428. 1944].

88. Lesley, J. W. and M. Shapovalov. The Riverside tomato. A new variety resistant to two wilt diseases. Seed World. March 26, 1937.

89. Lewis, A. C. and C. A. McLendon. Cotton variety tests for boll-weevil and wilt conditions in Georgia. Ga. Ent. Board, Bul. 46: 1–34. 1917.

90. Linford, Maurice B. A Fusarium wilt of peas in Wisconsin. Wis. Agr. Exp. Sta., Res. Bul. 85: 1–44. 1928.

91. Ludbrook, H. W. Pathogenicity and environal studies on Verticillium hadromycosis. Phytopath. 23: 117–154. 1933.

92. Mackie, W. W. Breeding for resistance of blackeye cowpeas to Fusarium wilt, charcoal rot, and nematode root knot. Phytopath. 24: 1135. 1934. [Abstract].

93. Mangin, L. La maladie des ocillets. Rev. hort. (Paris) 72: 205–208. 1900.

94. Miles, L. E. and T. D. Persons. Verticillium wilt of cotton in Missisippi. Phytopath. 22: 767–73. 1932.

95. Milikan, C. R. Wilt disease of flax. Jour. Dep. Agr. Victoria 43: 305–313. 1945.

96. Miller, J. C. Further studies and technic in sweet potato breeding in Louisiana. Am. Soc. Hort. Sci., Proc. 1938: 665–7. 1939.

97. ————. Further studies and technic used in sweet potato breeding in Louisiana. Jour. Hered. 30: 484–92. 1939.

98. ————, W. D. Kimbrough, B. M. Hughes and T. T. Ayres. Sweet potato breeding. La. Agr. Exp. Sta., Ann. Rep. 1941–2: 82–83. 1942.

99. ————, B. M. Hughes and T. T. Ayres. Sweet potato breeding. La. Agr. Exp. Sta., Ann. Rep. 1942–43: 78–79. 1943.

100. Moore, E. S. Wilt in Turkish tobacco. Farming in So. Africa 1: 380. 1927.

101. Mujica, F. Pathogenicidal de algunas del *Verticillium albo-atrum*. Rei. y Berth. Bol. Sanid. veg., Santiago 1: 7–20.

102. Mundkur, B. B. Resistance of American cottons to Fusarium wilt in India. Proc. Indian Acad. Sci. 3, 6: 498–501. 1936.

103. Neal, D. C. Cotton wilt: a pathological and physiological investigation. Ann. Mo. Bot. Gard. 14: 359–424. 1927.

104. Nelson, Ray. Verticillium sp. (?) wilt of peppermint. Pl. Dis. Rep., Supp. 50: 474. 1926.

105. ————. Production of mint species hybrids resistant to Verticillium wilt. Phytopath. 37: 18, 19. 1947 [Abstract].

106. ————. The specific pathogenesis of the Verticillium that caused wilt of peppermint. Phytopath. 37: 19. 1947. [Abstract].

107. ———— and L. C. Cochran. Breeding Fusarium yellows-resistant celery. Phytopath. 23: 25. 1933. [Abstract].

108. ————, G. H. Coons and L. C. Cochran. The Fusarium yellows disease of celery. Mich. State Col. Agr. Exp. Sta., Tech. Bul. 155: 1–74. 1937.

109. Norton, J. B. S. Tomato diseases. Md. Agr. Exp. Sta., Bul. 180: 102–114. 1914.

110. Ocfemia, G. O. and M. A. Palo. The relation of certain Philippine varieties of bananas to the wilt disease due to *F. cubense*. Philippine Agr. 15: 243–244. 1926.

111. Orton, W. A. The wilt disease of cotton. U. S. Dept. Agr., Div. Veg. Physiol. & Path., Bul. 27: 1–16. 1900.

112. ————. On the breeding of disease resistant varieties. Internatl. Conf. on Plant Breeding and Hybridization Proc. 1902. New York Hort. Soc. Mem. 1: 41–53. 1902.

113. ————. Some diseases of the cowpea. U. S. Dept. Agr., Bur. Pl. Ind., Bul. 17. 1902.

114. ————. A study of disease resistance in watermelons. Science 25: 288. 1907.

115. ————. On the theory and practice of breeding disease-resistant plants. Am. Breed. Assoc. 4: 144–156. 1908.

116. ————. The development of disease resistant varieties of plants. IV Conf. Internationale de Genetique, Paris. Comptes rendus et rapports. p. 247–65. 1911.

117. ————. Sea Island cotton. U. S. Dept. Agr., Farm. Bul. 787: 40. 1916.

118. POOLE, R. F. The stem-rot of sweet potatoes. Losses, sources of infection and control. N. J. Agr. Exp. Sta., Bul. 401: 32. 1924.

119. POPOVA, A. A. Diseases of tobacco, *Nicotiana rustica* L. Morbi Plantarum, Leningrad 18: 45–53. 1929. [Russian with English abstract].

120. PORTE, W. S. and H. B. WALKER. Pan America tomato. U. S. Dept. Agr., Cir. 611: 1–6. 1941.

121. ———— and F. L. WELLMAN. Development of interspecific tomato hybrids of horticultural value and highly resistant to Fusarium wilt. U. S. Dept. Agr., Cir. 584: 1–18. 1941.

122. PORTER, D. R. Breeding high-quality wilt-resistant watermelons. Cal. Agr. Exp. Sta., Bul. 614: 1–43. 1937.

123. ————. Pathogenicity of *Fusarium niveum* and the resistance of some watermelon hybrids. Phytopath. 20: 116. 1930. [Abstract].

124. ———— and HENRY A. JONES. Newly developed vegetable varieties for use in California. Cal. Agr. Exp. Sta., Bul. 646: 7–12. 1940.

125. ———— and I. E. MELHUS. The pathogenicity of *Fusarium niveum* (EFS) and the development of wilt resistant strains of *Citrullus vulgaris* (Schrad.). Iowa Agr. Exp. Sta., Res. Bul. 149: 125–183. 1932.

126. PRITCHARD, F. J. Development of wilt-resistant tomatoes. U. S. Dept. Agr., Bul. 1015: 18. 1922.

127. ———— and W. S. PORTE. The Break O'Day tomato. U. S. Dept. Agr., Cir. 218. 1932.

128. REINKE, J. and G. BERTHOLD. Die Zersetzung der Kartoffel durch Piltza. Unters. Bot. Lab. Univ. Gottingen 1: 1–100. 1879.

129. Report on the Agr. Exp. Stations 1946. U. S. Dept. Agr., Off. Exp. Sta. 90. 1946.

130. Rev. App. Myc. 12: 633. 1939.

131. RIKER, R. S. *Fusarium lateritium* v. *fructigenum* in relation to wilt of China aster. Phytopath. 26: 1085–6. 1936. [Abstract].

132. ———— and L. R. JONES. Fusarium strains in relation to wilt of China aster. Phytopath. 23: 29–30. 1933. [Abstract].

133. ———— and ————. Fusarium strains in relation to wilt of China aster. Phytopath. 25: 733–747. 1935.

134. RUDOLPH, B. A. *Verticillium hadromycosis*. Hilgardia 5: 201–253. 1931.

135. RYKER, T. C. Fusarium yellows of celery. Phytopath. 25: 578–599. 1935.

136. SALTER, R. M. Report of the Chief of the Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, 1946, U. S. Dept. Agr. 35. 1946.

137. SCHNIDER, H. Verticillium wilt of guayule. Phytopath. 34: 936. 1944. [Abstract].

138. ————. Surveys and observations of Verticillium wilt of guayule in California. Pl. Dis. Rep. 29: 615–617. 1945.

139. SCHROEDER, WILBUR T. and J. C. WALKER. Influence of controlled environment and nutrition on the resistance of garden pea to Fusarium wilt. J. Agr. Res. 65: 221–248. 1942.

140. Schuster, M. The nature of resistance of flax to *Fusarium lini*. Phytopath. 34: 356. 1944.

141. Shapovalov, M. and B. A. Rudolph. Essar—a new Verticillium wilt-resistant canning tomato. Seed World, Dec. 29, 1939.

142. ——— and J. W. Lesley. Wilt resistance of the Riverside variety of tomato to both Fusarium and Verticillium wilt. Phytopath. 30: 760–768. 1940.

143. Sherbakoff, C. D. Verticillium wilt of cotton. Phytopath. 19: 94, 1929; Tenn. Agr. Exp. Sta., Cir. 24: 2. 1929.

144. ———, P. R. Miller and D. M. Simpson. Use of liquid cultures for field inoculation of cotton. Phytopath. 34: 254–256. 1944.

145. Smith, A. L. The reaction of cotton varieties to Fusarium wilt and root-knot nematode. Phytopath. 31: 1099–1107. 1941.

146. ——— and W. W. Ballard. Wilt resistance in Empire cotton. Phytopath. 37: 436–7. 1947.

147. Smith, E. F. The watermelon disease of the South. Am. Assoc. Adv. Sci., Proc. 43: 289. 1894.

148. ———. The fungous infestation of agricultural soil in the United States. Sci. Am., Supp. 48: 1891–1892. 1899.

149. ———. Wilt disease of cotton, watermelon, and cowpea. U. S. Dept. Agr., Div. Physiol. & Path., Bul. 17: 1899.

150. ——— and D. B. Swingle. The dry rot of potatoes due to *Fusarium oxysporum*. U. S. Dept. Agr., Bul. 55. 1904.

151. ———. A Cuban banana disease. Science 31: 754–755. 1910. [Abstract].

152. Smith, F. E. V. Plant diseases in Jamaica in 1928. Ann. Rep. Dept. Agr. Jamaica 1928: 17–20. 1929.

153. Smith, T. E. and K. J. Shaw. Successful cross-inoculations with Fusarium from tobacco and sweet potato. Phytopath. 31: 21. 1941. [Abstract].

154. Snyder, W. C. and B. A. Rudolph. Verticillium wilt of pepper, *Capsicum annum*. Phytopath. 29: 359–62. 1939.

155. ——— and J. C. Walker. Fusarium near-wilt of pea. Zentralbl. Bakt. II. Abt. 91: 355–378. 1935.

156. ——— and H. A. Hansen. The species concept in Fusarium. Am. Jour. Bot. 27: 64–67. 1946.

157. Stakman, E. C., H. K. Hayes, Olaf S. Aamodt and J. G. Leach. Controlling flax wilt by seed selection. Jour. Am. Soc. Agron. 2: 291–298. 1919.

158. Stewart, F. C. Stem-rot disease of the carnation. Bot. Gaz. 27: 129–130. 1899.

159. Stoddard, D. L. Fusarium wilt of cantaloupe and studies of the relation of potassium and nitrogen supply to susceptibility. Trans. Peninsula Hort. Soc. 55(1941): 91–93. 1942.

160. Sturgis, W. C. Preliminary investigations on a disease of carnations. Conn. State Sta. Rep. 1897: 175–181.

161. Tharp, W. H. Sand-nutrient infection technique. Phytopath. 28: 206–9. 1938.

162. Thomas, H. E. Verticilliosis of strawberries. Phytopath. 21: 996. 1931. [Abstract].

163. ———. Verticillium wilt of strawberries. Cal. Agr. Exp. Sta., Bul. 530: 16. 1932.

164. Tims, E. C. The influence of soil temperature and soil moisture on the development of yellows in cabbage seedlings. Jour. Agr. Res. 33: 971–992. 1926.

165. ———. On the nature of resistance to cabbage yellows. Jour. Agr. Res. 32: 183–99. 1926.

166. Tisdale, W. H. Relation of soil temperature to infection of flax by *Fusarium lini*. Phytopath. **6**: 412–413. 1916.

167. ————. Flax wilt: A study of the nature and inheritance of wilt resistance. Jour. Agr. Res. **11**: 573–606. 1917.

168. ————. Influence of soil temperature and soil moisture upon the Fusarium disease in cabbage seedlings. Jour. Agr. Res. **24**: 55–86. 1923.

169. U. S. Dept. Agr., Pl. Dis. Rep., Supp. **86**: 55–56. 1935.

170. Uppal, B. N. Appendix K. Summary of the work done under the plant pathologist to the government, of Bombay Presidency, 1938–9: 203–211. 1940. [Rev. App. Myc. **19**: 641. 1940].

171. ————. Appendix K. Summary of the work done under the plant pathologist to the government, Bombay Presidency. 1935–36. 1937.

172. ————, Y. S. Kulkarni and J. D. Ranadive. Further studies in breeding for wilt resistance in cotton. I. Isolation of wilt-resistant types. II. A preliminary note on the genetics of wilt resistance in Indian cottons. Proc. Sec. Conf. Sci. Res. Wkrs. Cott. India, 31 pp. 1940 (?). [Rev. App. Myc. **20**: 256. 1941].

173. Valleau, W. D. Fusarium wilt of tobacco. Ky. Agr. Exp. Sta., Ann. Rep. **1933**, Part I: 22, 23. 1934.

174. ————, E. M. Johnson and Stephen Diachun. Tobacco diseases. Ky. Agr. Exp. Sta., Bul. 437: 60. 1942.

175. Van Beyma, F. H. Thoe Kingma. Uber einige formen von *Verticillium dahliae* Klebahn. Antonie van Leeuwenhock **6**: 33–47. 1939/40.

176. Van der Meer, J. H. H. Verticillium wilt of herbaceous and woody plants. Med. Landbouhoogesch [Wageningen] **28**: 1–82. 1925.

177. Vanterpool, T. C. Studies on Crown and Royal flax in flax-sick soil. Soil Sci. Agr. **24**: 268–270. 1944.

178. Verona, O., e A. Ceccareli. Su di una tracheomicosi dell Amaranto (*Amarantus tricolor* L.) produto du una specie di Fusarium a da Verticillium pathogeni. Phytopath. Zeit. **8**: 373–400. 1935.

179. Wade, B. L. Inheritance of Fusarium wilt resistance in canning peas. Wis. Agr. Exp. Sta., Res. Bul. 97: 1–32. 1929.

180. Walker, J. C. Resistance to Fusarium wilt in garden, canning, and field peas. Wis. Agr. Exp. Sta., Res. Bul. 607: 15. 1931.

181. ————. Diseases of cabbage and related plants. U. S. Dept. Agr., Farm. Bul. 1439: 35. 1927, rev. 1938.

182. ————. Disease resistance in vegetable crops. Bot. Rev. **7**: 458–506. 1941.

183. ————. Progress in combination of yellows and mosaic resistance with high ascorbic acid content in cabbage. Phytopath. **34**: 1012–1013. 1944. [Abstract].

184. ————, E. J. Delwiche and W. W. Hare. A major gene of resistance to near-wilt in pea. Phytopath. **34**: 1013. 1944. [Abstract].

185. ———— and W. W. Hare. Pea diseases in Wisconsin in 1942. Wis. Agr. Exp. Sta., Res. Bul. 145: 33. 1943.

186. ———— and W. C. Snyder. Pea wilt and root rots. Wis. Agr. Exp. Sta., Bul. 424: 16. 1933.

187. ———— and F. L. Wellman. A survey of the resistance of subspecies of *Brassica oleracea* to yellows (*Fusarium conglutinans*). Jour. Agr. Res. **37**: 233–241. 1928.

188. ———— and L. M. Blank. Fusarium-resistant Danish Ballhead cabbage. Jour. Agr. Res. **49**: 983–989. 1934.

189. Walker, M. N. A wilt-resistant watermelon for Florida. Fla. Agr. Exp. Sta., Bul. 288. 1936.

190. Wardlaw, C. W. Diseases of the banana and of the Manila hemp plant. 627 pp. 1935.

191. Welch, A. and I. E. Melhus. Wilt resistance in F_1 hybrid watermelons. Phytopath. 32: 181–182. 1942. [Abstract].

192. Wellman, F. L. and D. J. Blaisdell. Differences in cultural characters and pathogenicity in tomato Fusarium wilt. Phytopath. 29: 24. 1939.

193. Wenholz, H. Plant breeding in New South Wales. N. S. Wales Dept. Agr., Sci. Bul. 48: 43, 1935; and Bul. 51: 42, 1936.

194. White, R. P. Studies on Fusarium wilt caused by *Fusarium lycopersici* Sacc. Jour. Agr. Res. 34: 197–239. 1927.

195. White, H. L. On Verticillium wilt of the perpetual-flowering carnation. Jour. Pom. & Hort. Sci. 14: 216–226. 1936.

196. ————. Stem-rot and wilt of the perpetual-flowering carnation. Sci. Hort. 6: 86–92. 1938.

197. ————. On plant diseases, Rept. Exp. Res. Sta. Cheshunt, 1939: 28–38. 1940. [Rev. App. Myc. 19: 517. 1940].

198. Wickens, G. M. Wilt, stem rot, and die-back of the perpetual-flowering carnation. Ann. App. Biol. 22: 630–683. 1935.

199. Wilson, J. D. Celery yellows in Ohio. Ohio Agr. Exp. Sta., Bi. Bul. 168: 109–115. 1934.

200. Wilson, J. J. Pathological relationship between the host and parasite in varieties and strains of watermelons resistant to *Fusarium niveum* E.F.S. Ia. Agr. Exp. Sta., Res. Bul. 195: 106–152. 1936.

201. Wollenweber, H. W. Die Wirtelpitz-welkkrankheit (Verticilliose) von Ulme, Ahorn, und Linde USW Arb. biol. Abt. (Anst.-Reichanst) Berlin, 17: 272–299. 1929.

202. ———— und O. A. Reinking. Die Fusarien. 335 pp. 1935.

203. Yarwood, C. E. Increased yield and disease resistance of giant hill potatoes. Am. Potato Jour. 23: 352–369. 1946.

204. Young, V. H. Cotton wilt studies. I. Relation of soil temperature to the development of cotton wilt. Ark. Agr. Exp. Sta., Bul. 226: 50. 1928.

205. Zeller, S. M. Verticillium wilt of cane fruits. Or. Agr. Exp. Sta., Bul. 344: 25. 1936.

THE BOTANICAL REVIEW

VOL. XV JULY, 1949 No. 7

THEORETICAL ASPECTS OF GRAFTAGE

R. H. ROBERTS
University of Wisconsin

INTRODUCTION

The grafting of plants is a very ancient art. W. T. Chang (conversation, 4-16-45) reports that peach varieties were mentioned in Chinese writings as early as 1560 B.C. The presence of these varieties implies propagation by graftage. Graftage was discussed understandingly by Aristotle (384–322 B.C.), Theophrastus (372–287 B.C.), Cato (234–149 B.C.), Varro (116–27 B.C.) and others of their times. Working in the proper phase of the moon is sometimes a part of their recomended technique, and application of dung mixtures to the graft union was prescribed by some of the earliest writers. When we discarded this latter method we lost the benefit which the auxins contained in urine and manure mixture can contribute to the union of some stocks and scions (112, 208, 277). Su-lih reports in considerable detail suitable stocks for pears and plums in China about 500 A.D. (63).

The basic types of grafting and budding used today were described by Lawson in 1660 (227) and illustrated by Sharrock in 1672 (369). A list of 119 kinds and variations of grafting was described by Thouin in 1821 in his "Monographie des Greffes" (Cyclop. Amer. Hort., p. 661). This publication may have had great influence upon the extensive technical researches of Daniel 70 years later, especially since many of his results appear to have been largely conditioned by the grafting technique employed.

USES OF GRAFTAGE

The historical uses of graftage are so diverse and interesting that the taking of space to list the more common ones seems justifiable. Graftage is most commonly employed to reproduce plants which have desirable flower, fruit or foliage characters, or products (as drugs, resins [264] or latex [399]), but which do not come true from seeds, or do not reproduce readily from cuttings, layers, stolons, tips, *etc.* Other uses are: to change stature (either dwarfing [303] or invigorating); to change form, as by grafting a weeping variety upon a tall trunk; to adapt plants to wider climatic ranges, as by the use of hardy fruit tree stocks for more tender tops of varieties of better quality (116, 228, 273, 285, 411); to adapt desirable sorts to more soil types by using stocks of different soil preference (41, 110, 147, 324, 359); to hasten the fruiting of slowly bearing seedlings through grafting scions upon mature bearing plants (390, 434, 475); to change the variety of an undesirable kind planted by mistake (250); to grow several varieties, as of apples, upon a single stock, as in the urban yard; to "improve quality"; to form natural braces for weak crotches (240) (two branches twisted together may form a "natural graft") (105, 262, 282); to produce ornamental arbors; to provide a pollen source for self unfruitful varieties (most apples) or dioecious species (448); to immunize from diseases (145, 316), as a fire blight-free pear stock and root system (322); to evade insect pests (77, 104, 187, 188, 368), as some apple aphids (23, 157); to secure better root systems, as with roses; to avoid stocks which tend to sucker (66, 114), particularly plums and shrubs, as *Cotoneaster* and *Viburnum;* to repair rodent or disease injury or poor unions (124) by bridge grafting (191, 470) or inarching (141, 158, 269, 307, 308) with newly planted small trees; to transmit disease (virus variegation effects); to transmit the flower-inducing stimulus; to retard flowering; to aid pollination (468); to "create" new varieties; to study plant histology, transport and polarity (asymmetry) and to culture some "aborting" embryos.

Interest in graftage has developed along two rather separate lines, one being the technical aspects in the field of botany and the other the applied usages in agriculture, particularly horticulture. Both viewpoints have furnished a large amount of literature on theoretical aspects, but in general they have been published in different channels. Neither field has contributed much, aside from negative evidence, toward a solution of the important problem of the factors involved in the compatibility of stock and scion.

BOTANICAL CONTRIBUTIONS

Most of the papers on graftage by botanical workers have been concerned with either graft hybrids or the transfer of elaborated substances, particularly alkaloids, between stock and scion. Some papers have appeared on such subjects as inter-species or generic grafts, polarity of tissues, disease transfer, serological studies and, more recently, transfer of the stimulus-to-flower by grafting.

Successful grafting can be done on lower forms of plants, as algae (283), mosses and selaginella, as well as with the herbaceous monocots (267, 298) and dicots and woody gymnosperms and angiosperms.

Inter-grafting. Inter-grafting between species, genera, families or even orders is a topic of occasional interest. This interest seems to spring from an assumption that plants which are widely separated in their systemic classification have incompatible physiological differences. That some widely separated forms can be successfully grafted loses much of its interest when it is noted that it is common for closely related forms, as two apple varieties, to be uncongenial although the reciprocal graft is highly successful (428), or that two strains of a single variety show unlike compatibility to a single variety of stock (386, 422). Various similar cases of physiological incompatibility in closely related plants will be referred to in the later discussion of incompatibility.

Historically, inter-species as well as inter-genera grafts are regularly in use in horticultural practice with apples, pears, peaches, plums, roses, shrubs, and other ornamentals. The instances of such grafts in experimental work are far too numerous to justify being listed. Random examples are Daniel's experiments (82*a*), cotton and related species (24), olive on *Syringa vulgaris* (60), water cress on cabbage (83), *Chrysanthemum* on *Artemisia abrotanum* (113), tomato on different genera (204), *Humulus* (353), *Solanum* and *Iresine* (381).

Genetic differences appear to form no consistent basis for graft incompatibility (476).

Frequent cases of inter-family grafts are reported (478). When attempting to transfer the yellow's disease, Kunkel (222) had shoots from aster buds on peach which lived for as much as two months. Natural grafts are reported between families under forest conditions, especially root grafts (105, 282). A natural graft of a monocot and a dicot is recorded (166), but no vascular connection was formed in this union. The cases of dodder and mistletoe, while not considered as being grafts, are instances of natural unions of widely separated forms giving physiological exchange.

Obviously the technique of grafting plants even of widely different clasification is not of itself an essential, if even a valuable, procedure in a search for the basis of compatibility.

Graft Hybrids. The "graft hybrids" of botanical literature are not hybrids but chimeras. They are mixtures of tissue from two grafted plants, and arise at the point of union from an adventitious bud which forms from the callus tissues of both the stock and scion. These chimeras may appear naturally (17, 61, 72, 188, 457, 458), for example, after the frosting back of a scion (426) or from the heteroploid cells which are found in callus tissue (197), from cultural accidents which result in callus formation or from deliberate cutting off of a scion at the point of union to induce the formation of shoots from callus tissues at the union.* The two tissues may have one or more of three types of arrangement, appearing in the cross section view of the stem as wide or narrow (mericlinal) segments (sectorial chimeras), in layers with one so-called "parent" covering the other (periclinal chimeras) or with a mixed pattern (hetero or mixoclinal chimeras) (354, 357).

The periclinal nature of graft hybrids was deciphered by the cytological studies of Baur (19, 20) and has been verified by others (138, 362).

No instances of cell fusion have been demonstrated from a study of graft unions, although United States patent No. 2,048,056 was issued in 1936 with that "discovery" as a basis for producing hybrids. The failure of cells to fuse at a graft union clearly defines the impossibility of producing so-called sexual hybrids by grafting.

An historical discussion of graft hybrids seems to be unnecessary for two reasons. One is the numerous reviews or discussions, as by Swingle (405), Jones (196), Krenke (219), Winkler (461) and others (11, 49, 58, 127, 138, 169, 172, 195, 276, 329). A second reason for practically omitting an historical discussion of graft hybrids is that the work of Sansome (354), Jorgensen (197) and Crane (198), Baker (13), Blakeslee (31, 32) and Satina (356, 357) on heteroploid plants and the ready production of chimeras by agents such as colchicine (106) has removed the prime interest in the use of grafting as a method of securing chimeras. The determination that callus tissue contains heteroploid cells, even though in small numbers, makes understandable the erroneous argument of Strasburger (396) that plant chimeras are sex hybrids because of the chromosome numbers he counted in the callus tissue.

---

* Recent work with adenine to produce shoots from callus tissues suggests the possibility of using this technique to increase the numbers of chimeras which might be secured from graft callus (384*a*.)

Graft chimeras were of interest in a study of the origin of plant organs in the layers of the apex of the stem (224). For example, seedlings from graft hybrids are like the sub-epidermal "parent", since the origin of the gametic tissue is deep (354, 356). Material for such studies can be produced practically at will by the use of colchicine, making the more laborious and much less certain grafting technique no longer of much interest for that purpose (13, 19, 20, 138, 224, 358, 362, 396, 462, 463, 464).

Graft chimeras may also be produced by grafting split stems, as potato eyes (9, 173), lilac buds (181) and others (456, 457).

The numerous "hybrids" which Daniel and others have produced when grafting belong to another type of phenomenon. These interesting cases will be reviewed and discussed towards the end of this paper in a consideration of various problems of graftage which deserve further investigation.

Graftage and Polarity.—Grafting can possibly be used to learn more of the relations of anatomy to transport and growth. Vochting (388) found that cubes cut from beets did not unite properly when replaced in a rotated position. He showed that both longitudinal and radial orientation were important to good unions. Reversed polarity also gives "poor unions". This fact has been used to produce scion-rooted fruit trees (203); when a scion is set on an inverted piece root the union is poor and roots form from the scion above the union.

Poor growth is made by a graft when the scion is set in an inverted position (338, Fig. 28). Poor growth is also made by a yearling apple tree if it is ring-grafted with the ring of bark in an inverted position (342). It is believed to be significant that this dwarfish growth has a character different from that of the poor growth which follows girdling.

If these results involved conditions of transport they would bring into question the conclusion of Zimmerman (479) that "living cells do not determine direction of flow of materials". In the ingenious graft experiment from which he made his deduction the plant tissues were in normal gravitational positions.

White (454 and correspondence Dec. 7, 1943) doubts "that the grafting technique offers much promise for the study of tissue cultures themselves", although it is "an important step in studying the various plant tumors" (455). More recent studies by de Ropp report the successful grafting of callus tissue *in vitro* and the failure of inter-species grafts (106*a*).

Transport of Materials. The matter of the transfer of specific substances across a graft union has been frequently investigated for the past 50 to 60 years. A fair summary of the literature would seem to be that transfer depends upon whether the substance being investigated is naturally occurring in the scion and stock being used (215, 216). An illustration of this generalization is seen when artichoke and sunflower are grafted together, for the artichoke contains inulin whether it is scion or stock, and the sunflower characteristically contains starch (79, 436).

Examples of transfer are too numerous to list, particularly in cases of mineral substances, as potassium, magnesium (137), soluble solids (238) and boron (108). Transfer seems to be dominated more by the scion in some combinations and by the stock in others, but there would appear to be no general rule involved. A particularly interesting situation is reported by Hofmann (178) and Richmond (328) that seedlings from bean grafts can be cross-inoculated, whereas the graft components can be successfully inoculated only with their specific bacteria.

Reports of failure to secure transfer of substances are especially numerous. Some examples are: hydrocyanic acid did not appear in fruits of cherry or cherry laurel (72); starch was not present in cabbage (which stores fat) when grafted with turnip (79); anthocyanin difference of bean varieties remained the same after grafting (178); likewise, red and white beets did not change color (24*a*); the latex content of *Hevea* remained quantitatively different (179), as did the sugar content of beets (285) and the alkaloid composition of *Derris* (194); molybdenum was present in the apple stock Malling IX but not in the Lane's Prince Albert scions (334); the red color of the Hopa crab wood remains specific (341–343, 469); and grafting of male and female plants of bittersweet (*Mercurialis annua*) did not change the sex (467).

Much attention has been given to another case of transfer, that of the alkaloids of the Solanaceae. The long argument seems to have been brought to a close by the discovery that nicotine is formed in the roots (98–101, 363). Peacock *et. al.* (294) state that Strasburger's (393) assumption that alkaloids originate in the leaves delayed progress 50 years until in 1941 Krajevoj and Nechaev (218), Hieke (168) and Dawson (97) showed that it is synthesized in the roots.

Some alkaloids are formed in the leaves, as anabasine by *Nicotiana glauca* (100), and by the leaves of the stock in lupine grafts (274). Transfer depends upon whether they are naturally occurring in both graft components.

√ The failure of materials foreign to a specific plant to pass a graft union raises the question, which will be discussed later, as to the significance of graft effects.

Transfer of the Stimulus-to-Flower. One of the newest uses of grafting involves transfer of a material, the unidentified substance which induces flowering (51, 71, 143, 162, 220, 270–272, 284). This technique consists of approach-grafting a flowering plant (the donor) on to a non-flowering plant of the same species (the receptor) with the expectation that the latter will be induced to flower. Several known factors influence or determine the success of the operation: physiological contact is needed, the leaf area on the receptor should be reduced, the flowering habit of the species is important and inter-species grafts fail.

Moshkov (275) reports that *Perilla* grafts must be in "physiological contact" for a continuous period of ten days to effect a transfer of the flowering stimulus. The Withrows (465) found that a four day contact is needed with *Xanthium* grafts, and Heinze, Parker and Borthwick (162) state that a similar period of contact is requisite to a successful transfer of induction with Biloxi soybean grafts. The passage of the induction stimulus through a "diffusion membrane" would be contrary to the above reports. The case reported (143) may be questioned, since the material used was lens paper. This is sufficiently porous to permit cell contact of the callus tissues through the open spaces in the unevenly and loosely woven fibers (465).

It is common experience that the presence of leaves on the receptor plant greatly delays or prevents its induction (52, 54, 143). In *Xanthium* removal of the fruits from the donor also favors transfer.

Successful induction by grafting seems to require plants which have a systemic blossoming habit (345). Plants which have only terminal blossoms, as poinsettia, Knondyke cosmos, *Salvia* var. Harbinger and stock var., Christmas Pink, will not induce by grafting or by photoperiod treatment of one of the branches.

Grafts between species made to induce flowering have been reported to be unsuccessful (162, 284). One seems obliged to defer judgment on some experiments conducted with *Hyoscyamus niger* (258), since this species, or at least its strains, appears to make a much stronger reaction to temperature than to photoperiod. Interpretations of data on this plant, which are available to date, might prove to be misleading.

Single mature leaves from flowering plants will induce another plant following grafting, if the species selected and other conditions are favorable (51, 143, 270).

Disease Transfer. Grafting provides a successful means of transferring virus diseases (144, 221), especially when they are not easily carried in sap injections or by feeding insects (175, 252, 449). Some transfers of peach yellows are affected in as short a period as five days (222), although at other times "within ten weeks" (408). One disease is said to be transmitted by grafting, but not by budding (175). The ease of transfer of disease by grafting is a serious hazard with some horticultural crops (38, 200, 315, 321), especially when latent viruses are encountered (261, 312).

Variegations due to virus conditions are transferred by grafting (18, 64), and some "chimeras" may be similarly involved (34).

Resistance to graft transfer is genotypic in at least some cases (33).

Serological Studies. The idea that the compatibility of plants for graftage can be determined by such a technique as the precipitin reaction has been rather extensively investigated (67–70, 125, 131, 210, 212, 214–216, 305, 331, 376, 378, 379, 453). It is stated that some Solanaceous species acquire an immunity against certain antigenic agents of the other graft component, but definite evidence that plants are able to form antibodies seems to be lacking. Others report that there may be no change in immunity, or that in some combinations even a loss of precipitins occurs (210, 212). The problem is complicated, since pathological conditions may be interpreted as evidence of antibody action (215). Mineral accumulations may also give an incompatibility condition comparable to some antibody activity (378). It seems that a precipitin type of test has little value in studies of compatibility (305 and correspondence March 29, 1943, 453).

AGRICULTURAL CONTRIBUTIONS

The studies on graftage by workers in agriculture have largely been in connection with a search for solutions to practical problems, such as those of hardiness, soil adaptation and control of stature. They have, however, yielded data of theoretical importance and contributed to a better definition of the nature of graft influence. The practical phases of graftage will not be reviewed here; these have been well discussed in a number of bulletins from Agricultural Experiment Stations and in similar publications, particularly upon propagation (1, 59, 288, 364, 406, 471).

Numerous papers have been written upon the suitability of different stocks for various fruit crops, as apples (116, 146, 149, 201, 223, 245, 371, 391, 425), pears (62, 148, 414, 416), cherries (42, 47, 135, 183, 193, 254, 310), plums (2, 75, 151, 153, 154, 423), peaches (192, 311), apricots (386), quince (424), grapes (25, 35, 126, 236, 241, 323, 410, 431) and citrus (5, 16, 46, 56, 140, 190, 269, 309, 360, 383, 407, 447).

Shrubs are extensively grafted (184, 349, 432, 450, 472) as are also evergreens (263).

Nut groves are commonly planted with grafted trees (12, 26, 319, 320, 384). The tung tree (246) and even some cottons (24) are being propagated by graftage. Interest is increasing in the graftage of tropical fruits and plants, as cacao, coffee, mango and rubber yielders (50, 118, 248, 287, 291, 301, 351, 359).

Graftage is so old an art that little new in techniques appears, although frequent titles list "new" methods. Two ideas do particularly deserve mention. These are the employment of a special adhesive tape to restrict callus growth and prevent galls on bench grafted apples (330) and the use of cotyledons to nurse avocado embryos (412). It is not argued that either of these would be found to be entirely new if sufficient search of the literature was made. They are, however, examples of new procedures in practical propagation.

Compatibility. The question of compatibility of stock and scion is in some respects the largest question of graftage. As to the solution of the problem, the situation remains much the same as Argles stated it in 1939 (8). "There is unfortunately as yet no means of determining in advance of experience what stocks and scions will be compatible or incompatible nor with what severity any incompatibility will be manifest".

We are not concerned with shades of meaning which may be expressed by words somewhat synonymous to compatibility, as congeniality or adaptability (186). By compatibility it seems best at present to refer to the long time success of a graft for economic, esthetic or scientific purposes. Anything less is incompatibility, partial compatibility, delayed incompatibility or some similarly expressed condition. This viewpoint considers compatibility as any inter-influence between stock and scion and not merely effects arising directly from the union.

Rough unions or unequal growth in diameter of stock and scion have been blamed for incompatibility conditions (37, 102, 165). It is common experience, however, to see successful grafts with such "defects" (79).

It seems a safe conclusion to state that dwarfness as a result of grafting is not due to incompatibility (372).

The complex nature of the problem of finding an answer to the question of compatibility may be seen from the following list of phenomena which are examples of the difficulties involved:

a) The success of apple piece-root grafting changes with the age of the piece-root used as the stock. Roots from yearling seedlings give complete success; roots from the same lot of seedlings if two or three years old commonly yield no more than 10 percent of successful unions. Probably cambial activity is involved (130).

b) Some plants, as Bechtel crabs, propagate successfully when budded, but not when grafted.

c) One stock and scion combination may be highly successful and the reciprocal combination wholly worthless (28, 102, 164, 189, 333, 344).

d) Strains of a single apple variety may show different reactions to the same stock (387, 421, 422).

e) "Delayed" incompatibility of what may have been considered a successful graft combination for even several years is frequently reported (14, 150, 182, 290, 327, 373). This situation bcomes particularly serious in cases like the development of blossom end rot of pears on certain stocks as late as the time peak production is approached (95, 163).

f) Some plants should be grafted at the collar of the stock and not below it (375).

A number of factors have been named from time to time as important to compatibility:

a) The statement that etiolated plants can not be successfully grafted (80) is not in line with the report of grafting pea seedlings in the dark (452) or the use of grafting in studying the development of tissues in *in vitro* culture (455).

b) It has been found that it is sometimes an advantage to cut the scion so that the top bud (with its apical dominance) is in line with the point of callus union to take advantage of the more rapid flow of sap in a vertical line. This benefit seems to be important only when growth conditions are not good (337, 401), but of little value under optimum conditions for the new graft to grow (123).

c) Ease of water conductivity through the union has been suggested as one factor in compatibility of stock and scion (63, 139).

d) Interrupted nutrition is a common suggestion used to explain incompatibility (65). This condition appears to be met with especially in cases where a disease-resistant stock is involved (253).

e) Incompatibility has been found when there is a marked difference in the starch content of the stock and scion (367). In some cases the deduction was faulty because one or the other component was a storer of fat or inulin instead of starch.

f) Failure of the reciprocal graft of geranium on tomato is explained as due to exosmosis to the acid geranium (466). A similar situation involved the osmotic pressure interaction of the albuminoids (332).

g) Protein specificity has been reported to be a factor in the success of a union (377, 380).

h) Virus disorders may enter into some cases of "chimeras" and "incompatibility" situations (34).

i) The scion was girdled by one case of disorder at the union in a walnut graft (365).

j) A compatible union may be secured by retaining some branches on the stock when top-working apples (257).

k) The top-working of cherries was successful for one worker when done in late September, but not in the spring (237).

l) Climate is blamed for the success or failure of grafts in different geographic locations. Examples are the successful grafting of Duchess d'Angouleme pear on quince in England and the United States and its failure in Germany, or the failure of sour orange as a stock in South Africa and Java but its success elsewhere. In the latter case the interesting explanation has been offered that the difficulty is due to the deficiency of rare elements and not to climate (16, 57, 120, 247).

m) Compatibility of stock and scion appears not to be due to the parentage relations of stock and scion (225), but is strongly related to anatomical characters (278).

The nature of the union has received much consideration (40, 41, 48, 76, 88, 122, 199, 211, 251, 259, 281, 286, 304, 306, 355, 394, 446). These studies are generally of more interest to students of anatomy than as contributions to the theories regarding graftage. Typical considerations are ontogeny of tissues at the union (76), presence of plasmodesma (48), tyloses (88), cell inclusions (281), cytoplasmic content (394), origin of callus (211) and tannin formation (251). There is, of course, no fusion of cells at the callus union.

A disappointing feature of the studies on compatibility is that no basis has been found upon which to forecast the success of a proposed stock and scion combination. The nearest to a guide is that the relative season of growth is important to the success of a graft. Baltet (14) states that the scion and stock should have the same starting season (perennials) but that if there is a difference, the scion should be later. The hope that this might be a rule for practice disappears if we accept Lindemuth's report that poor trees can be expected if a late starting sort is grafted on an early starting kind. The reciprocal graft is successful. Our observation with apples has been that an early fall (wood) maturing variety grafts well on an early, but not on a late maturing kind. The late sort will succeed on an early or a late one (344). More examples are needed to give this "rule" validity.

Stock Influence. The large item of interest in graftage from the agricultural standpoint has been stock influence. Only the theoretical aspects of this problem will be reviewed. The practical implications can be obtained from a number of publications (7, 36, 44, 62, 77, 103, 126, 140, 149, 236, 241, 269, 314, 316, 323, 326, 338, 339, 340, 350, 370, 383, 384, 407, 420, 428, 447). Dwarfing stocks have been a special center of interest (27, 29, 55, 347, 398, 417, 440). The argument has also been advanced that clonal stocks produce a more uniform orchard population than seedling stocks. Much evidence has accumulated recently showing that trees are not appreciably more uniform when grown on vegetatively propagated stocks than on seedlings (6, 392, 397, 402, 429). An explanation of why this is so will be delayed until after the theoretical nature of stock and scion influence is reviewed.

One of the commonest theories used to explain the fact that a scion variety grows differently on different stocks has been that of altered mineral uptake. In this connection it has been repeatedly shown that the same variety on different stocks "requires" different fertilizer applications (3, 121, 155, 174, 202, 235, 295, 334, 335, 430, 437). Attention has also been called to the poor flow of water past the unions with a dwarfing stock (at least with some scion varieties [444]).

An important advance in the understanding of stock effects with the apple was the finding that this effect is localized in the stem portion of the stock (338, 404). That is, a scion variety responds differently when grafted directly upon a piece of root than upon the stem of a seedling or upon a rooted stem cutting. It has also been shown that long pieces of apple roots planted so that a portion is above the soil and in the light, permitting development of green pigment and assumption of "stem characters", produced effects similar to those of stocks which were grafted on the stems (unpublished data.) These observations suggest that stock effects are problems of transport.

Further evidence that transport may be responsible for some stock effects is supplied by the results with ring grafts. A typical stock influence can be produced in apples by grafting a ring of bark upon a yearling tree (341–343, 469). In this connection it appears that the particular influence upon transport involves the carbohydrate materials (313, 323, 388). The data of Colby support this probability (74). It may be significant that the deduction that "root stock effects cannot be explained on the basis of the chemical characters examined" was arrived at without analyzing for carbohydrate content (445). The sugar content of citrus fruits did not vary with some stocks (382).

The strong growth of one apple variety (*e.g.* Fameuse) on a stock (Malling IX) which is very dwarfing for another variety (as Whitney) becomes understandable when the carbohydrate nutrition is considered (344). It is common experience for stocks to have opposite effects upon the vigor of different varieties (176, 242, 427).

Some stock effects provide interesting theoretical items:

a) Apple trees on dwarfing stocks usually grow as vigorously as those on standard stocks while in the nursery row (418).

b) Time of blossoming is often changed by stock influence (152, 443) as are also fruit content (177) and maturity (256, 292, 346).

c) The so-called invigorating effect of a stock (171, 302) raises an interesting question as to whether the growth of a scion variety has really been improved or whether a certain stock merely provided an opportunity for optimum growth. For example, a "southern" apple variety, as York, grows poorly in the nursery in the climate of a northern latitude when grafted on seedling piece roots; but some stocks, particularly a semi-dwarfing or dwarfing kind, markedly improve the growth of this variety in northern locations.

d) The production of "vegetative hybrids" (10, 204), "new species" (91, 92), *etc.* by graftage is a subject to be discussed later.

e) Graftage may change the anatomical structure of a scion (85), but this is probably not different from a condition produced by changes in nutrition.

f) The marked variation in vigor of apple grafts in the nursery row (123, 337) is a very interesting unsolved problem. It is obviously in part a scion source question and may be closely associated with the reason why spurs or branches along a one-year shoot are so variable in length.

g) The "permanence of size differences" which arises in the nursery and continues in the orchard (361) can be expected to disappear if the planting is carried at an optimum instead of a marginal cultural level. It is common experience for small nursery trees to produce fine orchards under proper cultural treatment.

h) The climatic range of annuals (228, 385) or perennials (115) can be extended by grafting.

i) The effect of stocks upon susceptibility (268) or resistance to disease had best be investigated from the viewpoint of scion-host effect as well as stock influence.

j) A white-flowered hibiscus which does not root readily can be made easy to root from cuttings after grafting it upon an easy-rooting red variety (433).

The observation of Zebrak (478) that the "physiological changes (in the scion) are not directly due to grafting" is probably well taken. This possibility will become more clear after the review of the topics of scion influence and double-working is completed.

Scion Influence. Historically, the fact that the scion may exert a marked influence upon the amount, character or color of the root system of the stock has been well known for at least a hundred years (128). This fact was largely lost sight of in the active interest in apple stock effects in England in this century (146, 147, 149). Since 1927 (337) scion influence, as well as stock influence, has again been given attention (15, 142, 289, 338, 389, 400, 402, 404, 409, 439), even with herbaceous forms (249).

In the same fashion that the stem portion of the stock can be said to be the seat of stock influence, the stem of the stock is likewise the factor which most often determines the expression of scion influence. When a scion variety (apple) is budded or grafted upon the stem of a seedling or clonal stock (rooted stem), especially a dwarfing stock, little to no evidence of scion influence upon the root system of the stock is apparent. On the other hand, if the scion is grafted upon a piece of root (without stem) the subsequent development of the roots is markedly affected in amount, type, anatomical character, or even health by the scion variety (21, 22, 338–340, 400, 404, 411). Botanically, the influence of the scion upon the anatomical structure of the roots (338) is particularly interesting.

Various attempts to obtain more productive orchards by propagating from high-yielding trees have given conflicting results (78, 96, 300). An understanding of this question is more concerned with the relations of vigor and character of growth to blossom induction than to a possible grafting influence. The marked difference in physiological condition of scions from biennial varieties in alternate years is enough of a factor to produce differences in growth of grafts to sometimes, under some conditions, give evidence that productivity is "transmissible". Under other growing conditions the physiological difference of the scions would be eliminated very soon and the precosity difference lost.

Changing the color of potato tubers by grafting on a variety of a different color (265) was not obtained by the intergrafting of the red varieties Triumph and Red Warba with the white varieties Rural New Yorker and Katahdin (unpublished data). However, the marked effect which cultural treatments, as photoperiod and temperature, have upon the coloring of some varieties of potato tubers suggests the possibility that scion influence might with some varieties and under some growing conditions alter tuber coloring.

Scion influence may increase the cold hardiness of a stock (117), obviously through maturation phenomena.

Double-working. The demonstration that the scion variety dominates the root development (337, Fig. 11) when the scion is grafted upon a root but may have little influence if a portion of stem intervenes, as in seedlings or rooted cuttings (337, Fig. 12), led to the deduction that stock effects are localized in the stem portion of the stock. This raised the question of the practicability of producing stock effects by double-working instead of using the more expensive operation of vegetative propagation to produce stocks; why not graft the stock variety upon a piece-root and then graft or bud the scion variety upon the stock graft? It has been clearly demonstrated that stock effects can be produced with this technique (28, 39, 45, 136, 167, 171, 205–207, 243, 244, 255, 338, 415, 419, 441, 442). Practically this method of propagation is used to secure resistance to cold (117); to diseases, as fire blight of pears, collar rot of apples, cherry buckskin disease and cherry gummosis; to insects like wolly aphis; and to nematodes.

Intermediate stocks can be used to effect a successful stock and scion combination which is incompatible when grafted directly together (134).

The effects of double-working upon productivity are similar to those from using clonal stocks from vegetative propagation. Also, similar incompatibilities and differences in responses of reciprocal grafts are experienced (156, 344, 452).

"Neutral" Stocks. When working with intermediate stocks it became apparent that some stocks do not alter scion influence upon root development (344, Fig. 9).

Also, some clonal stocks yield to scion influence (344, Fig. 7). This failure to show typical stock effects was described as being due to a "neutral" stock. This phenomenon, whatever it should be called, makes understandable some reports of condradictory results from double working as well as from the use of clonal stocks (15, 230, 231, 344, 348, 439).

Several interesting theoretical items are suggested by the neutral stock situation:

a) The uniformity of trees in an orchard propagated upon seedlings is obviously due to the failure of the miscellaneous collection of seedlings to exert stock influence. No definite measurement has been made of the percentage of apple seedlings commonly used as stocks in the United States which show a positive stock effect. It is doubted that it would prove to be as high as two or three percent, at least in trees sold after grading for nursery sale. Inspection of trees in the nursery row reveals relatively few cases of incompatibility or obvious stock influence.

b) The usual vigorous growth of nursery and young orchard trees on what appear to be neutral (non-positive) stocks indicates that good vigor is not due to an "invigorating" effect of the stock but merely to the lack of a dwarfing effect.

c) A given stock may show a lack of influence (at least dwarfing influence) upon one scion variety, but have a marked effect upon another one.

We may now consider the theoretical viewpoints of the English reports with a possibility of better understanding and appraising them. The selection and classification of the various vegetatively propagated stocks for apples yielded strains which greatly helped in the commercial production of trees with different vigor habits and resultant adaptations to different practical uses (146). Along with this work the argument developed in England that trees on a clonal stock would be uniform in vigor and productivity. This was important because of the usual variability of orchard trees budded on the seedling stocks regularly in use there. Scion influence was considered to be unimportant. In time it became apparent that the theoretical expectations were not all realized. Some stocks behaved differently on different soils (147): some varieties, as Cox's Orange Pippin and Lane's Prince Albert, showed "reverse vigor" on two stocks; "delayed incompatability" was encountered; and some instances of scion influence were found (149).

It appears possible that the recorded uniformity of trees propagated at the East Malling Station was as much due to the influence of the heading back of the yearling trees too near the ground and the practices followed in grading the stocks, as it was to the use of clons as stocks, or more so. (338, Chart II, and Fig. 26).

The much greater variability of trees propagated on seedlings in English orchards leaves us with the question of whether the European seedlings are more positive in their effects than the American ones. Since both countries formerly used "French seedlings" as stocks, cultural differences, as the age at which grafting was done, appear still to be a factor in the unlike performance in the two countries.

One other theoretical consideration affects the deductions from experimental work, especially in England. This is the observed but not too well measured probability that the commercial varieties grown in England have a narrow physiological range. That is, from climatic, geographic or other limitations, the varieties are much alike physiologically as compared to American varieties of wide temperature, seasonal, climatic and geographic adaptation. It was seen in a trial of eight English varieties that the widely different scion effects, such as are secured when American kinds—Tetofsky, Whitney, Fameuse and Winesap—are used as scions, were not found (unpublished). The root systems on piece-root trees when one year old were much alike except in volume for the following kinds: Beauty of Bath, Bramley's Seedling, Grenadier, Grieve, Lane's Prince Albert, Newton, Tyler's Kernel and Worcester.

DISCUSSION

The failure of stock and scion to exchange specific substances not common to each, casts doubt upon graftage being a means of producing any physiological condition not to be duplicated by the use of environmental factors or combinations of factors, as geotropism (for orientation), temperature, photoperiod, shade, girdling, defoliation and fertilizing. If not, grafting is then only a technique to be used to produce experimental material and not a special problem to be studied theoretically. Has a stock a specific influence or it is merely another environmental factor? Winkler maintained that specific influence from grafting had not been proven (459), and Griffen (133) considers graftage effects not different from "nutritive variations". Zebrak has similarly concluded that physiological changes resulting from grafting are not due to grafting "as such" (478).

If this apparently correct view be taken, we have the problem of rationalizing results as reported by Daniel (79–92) and others (10, 43, 107, 160, 161, 170, 211, 213, 217, 265, 317, 318). They state that grafting produced such effects as altered fruit shape and flavor, changed seedling development, reduced pollen fertility, and "created" new varieties. If the graft union is looked upon as an environment, its effects would vary from very good (compatible union) to very poor (quite incompatible). It is common experience for plants to show variability and abnormalities under unfavorable environments which permit unusual genetic expression. In a review of environmental influences Hiesey states further that "recent developments in the experimental alteration of chromosome number, the causing of chromosomal rearrangements, and the induction of mutations through temperature effects [296], aging of seed [278], radiation [129], drugs and growth hormones [30, 109, 132, 280], now make it clear that external factors may produce rearrangements or changes in the germ plasm. While there is no acceptable data at the present time to show that natural environments directly induce changes that become fixed in heredity, the possibility of externally induced hereditary changes through some action on the germ plasm must now be admitted". A poor graft union provides an environment suitable for the production of plant variability. At least that seems to be the view which should be taken of "questionable" results of grafting until more is known of the effects of environment upon the expression of plant characters.

The effect of dwarfness upon the productivity of a fruit tree has made it an interesting perennial question. The real problem is, however, from the standpoint of fruit production, not the nature of dwarfness, but instead, one of the physiology of blossom induction (403).

When planning experiments to observe stock and scion relations, scions from varieties of a wide physiological range should be used. A common fault of most graftage experiments is to use scion varieties which are too much alike physiologically. This error comes principally from using only commercially important varieties. These are all adapted to the locality of the experiments, and naturally are much alike from the nutrient standpoint.

A list of pertinent problems in connection with graftage is much the same as it was nearly 20 years ago (337–338). Some of the problems are:

a) The relative time of wood maturity appears to be the only tangible factor upon which to predict the success of a stock and scion combination within a species. Is this relation, when it prevails, a matter of physiology or morphology or both?

b) Is transport a factor in "neutral" stocks?

c) Is cambial condition involved in the development of incompatibility as seedling roots grow older?

d) Why do reciprocal grafts react differently?

e) Does the variability of buds along a scion contribute to the variability in the growth of grafts in the nursery row?

f) Why do plant size differences, which are of physiological origin, tend to persist in the orchard sometimes for years?

g) Is the "invigorating" effect of a stock merely a case of the absence of dwarfness?

h) Is the imparting of hardiness by a stock primarily a matter of maturity?

i) Has graftage any specific effect, or is it only one of the many environmental factors?

SUMMARY

Since graftage involves a stock and a scion, when grafting, budding or making a transplant, consideration of the theoretical aspects of this operation is much concerned with compatibility. Neither technical studies in botany nor practical experience in agriculture have contributed a satisfactory answer to the question of how to predict a successful graft combination.

The frequent failure of grafts between strains of a single clon detract from interest in why grafts between widely unrelated species are generally unsuccessful.

Interest in graft hybrids has lost its former intensity with the demonstration that these are chimeras and that heteroploid tissues which give rise to chimeras can be induced by substances like colchicine.

The failure of elaborated or unelaborated materials to pass a graft union unless they commonly occur in both the stock and scion raises the question as to whether graftage can have a specific effect or is only one among the list of environmental factors.

Workers in agriculture commonly emphasize stock influence in graftage, particularly dwarfing effects. Stock effects have been

found in apple to be localized in the stem portion. In fact, stock effects can be produced by a ring of bark. Obviously, dwarfing effects are in such a case a matter of altered transport.

The so-called invigorating effect of a stock may be merely the absence of some dwarfing factor.

Scion influence upon root development is often very striking. As in stock effects, scion influence is modified by the stem portion of the stock.

Clonal stocks do not guarantee a more uniform size of orchard trees than some seedling stocks. Cultural operations in the nursery are an important factor in tree uniformity.

Many theoretical questions in graftage remain unanswered.

BIBLIOGRAPHY

1. Alderman, W. H. Grafting and budding. Minn. Agr. Exp. Sta., Spec. Bull. 151. 1932.
2. Amos, J. and Witt, A. W. Plum rootstocks: their varieties, and influence upon cultivated varieties worked thereon. Jour. Pom. & Hort. Sci. 7: 63–99. 1929.
3. ————, *et al.* The nutrition of fruit trees. II. The response of apple trees on known rootstocks to applications of complete fertilizer. Ann. Appl. Biol. 17: 657–674. 1930.
4. ————, *et al.* Studies in incompatibility of stock and scion. I. Information accumulated during twenty years of testing fruit tree rootstocks with various scion varieties at East Malling. East Malling Res. Sta., Ann. Rep. 1936: 81–99.
5. Andrade, Ed. Navarro De. (Grafting stocks for citrus). Bol. Agr. (São Paulo) 31: 869–884. 1931. (Biol. Abst. 7: 21865. 1933).
6. Anthony, R. D. and Yerkes, G. E. The influence of clone roots on the variability of young apple trees. Proc. Am. Soc. Hort. Sci. 25: 169–171. 1928.
7. ————, *et al.* Orchard tests of Mazzard and Mabaleb cherry understocks. Proc. Am. Soc. Hort. Sci. 35: 415–418. 1938.
8. Argles, G. K. A review of the literature on stock-scion incompatibility in fruit trees, with particular reference to pome and stone fruits. Imp. Bur. Fruit Prod., East Malling, Tech. Commun. No. 9: 5–115. 1937.
9. Assejeva, T. Bud mutation in potato. Bull. Appl. Bot., Genet. & Pl. Breed. 27: 135–217. 1931.
10. Avakian, A. A. and Jastreb, M. G. Hybridization by grafting. Jarovizaloiia 1: 50–77. 1941. (Biol. Abst. 16: 3552. 1942)
11. Babcock, E. B. and Clausen, R. E. Genetics in relation to agriculture. pp. 356–371. 1927.
12. Bailey, J. E. and Woodroof, J. G. Propagation of pecans. Ga. Agr. Exp. Sta., Bull. 172. 1932.
13. Baker, R. E. Induced polyploid, periclinal chimeras in *Solanum tuberosum*. Am. Jour. Bot. 30: 187–195. 1943.
14. Baltet, C. L'art de greffer. 1902. [Eng. trans. 1923].
15. Barker, B. T. P. The relations of scion and rootstock. Agr. & Hort. Res. Sta., Long Ashton, Ann. Rep. 1927: 32–41.
16. Batchelor, L. D. and Webber, H. J. Progress report of lemon rootstock experiments. Cal. Citrograph. 24: 160–161. 1939.

17. Bateson, W. Studies in variegation. Jour. Genet. 8: 93–99. 1919.

17a. ————. Root cuttings and chimeras. Jour. Genet. 11: 91–97. 1921.

18. Baur, E. Zur Aetiologie der infectiösen Panachierung. Ber. Deut. Bot. Ges. 22: 453–460. 1904.

19. ————, Das Wesen und die Erblichkeitsverhältnisse der "Varietätes albomarginatae Hort." von *Pelargonium zonale*. Zeits. Ind. Abs. Vererb 1: 330–351. 1909.

20. ————, Pfropfbastarde. Biol. Cent. 30: 497–514. 1910.

21. Beakbane, A. Anatomical studies of stems and roots of hardy fruit trees. III. The anatomical structure of some clonal and seedling apple rootstocks stem- and root-grafted with a scion variety. Jour. Pom. & Hort. Sci. 18: 344–367. 1941.

22. ———— and Thompson, Eleanor C. Anatomical studies of stems and roots of hardy fruit trees. II. The internal structure of the roots of some vigorous and some dwarfing apple rootstocks, and the correlation of structure with vigor. Jour. Pom. & Hort. Sci. 17: 141–149. 1939.

23. ————, *et al.* Preliminary selection of apple rootstocks immune from wooly aphis based on certain anatomical differences in root structure. East Malling Res. Sta., Ann. Rep. 1940: 32–36.

24. Beckett, R. E. Budding and grafting trials with cotton and related plants. U. S. Dept. Agr., Circ. 267. 1933.

24a. Biffen, R. H. Note on some grafting experiments. Ann. Bot. 16: 174–176. 1902.

25. Bioletti, F. T. *et al.* Phyloxera-resistant stocks. Cal. Agr. Exp. Sta., Bull. 331. 1921.

26. Blackman, G. H. Topworking pecan trees. Fla. Agr. Exp. Sta., Bull. 170. 1924.

27. Blackman, V. H. Some physiological considerations in horticulture. Proc. I. Imp. Hort. Conf. II. 37–41. 1930.

28. Blair, D. S. Rootstock and scion relationships in apple trees. Sci. Agr. 19: 85–94. 1938.

29. ————. Present status of the apple rootstock and double-working trials in eastern Canada. Sci. Agr. 20: 150–154. 1939.

30. Blakeslee, A. F. The present and potential service of chemistry to plant breeding. Am. Jour. Bot. 26: 163–172. 1939.

31. ————. Effect of induced polyploidy in plants. Am. Nat. 75: 117–135. 1941.

32. ———— and Avery, A. B. Methods of inducing doubling of chromosomes in plants. Jour. Hered. 28: 393–411. 1937.

33. Bond, T. E. T. *Phytophthora infestans* (Mont.) de Bary and *Cladosporium fulvum* Cooke on varieties of tomato and potato and on grafted Solanaceous plants. Ann. Appl. Biol. 23: 11–29. 1936.

34. ————. Disease relationships in grafted plants and chimeras. Biol. Rev., Cambridge Philos. Soc. 11: 269–285. 1936.

35. Bonnett, L. O. Phylloxera resistant vineyards. Cal. Agr. Exp. Sta., Circ. 288. 1925.

36. Booth, N. O. Some investigations in grafting. Proc. Am. Soc. Hort. Sci. 10: 144–149. 1913.

37. Borzini, G. (The cause of vine blight). Ball. R. Staz, Patol. Veg. 18: 137-146. 1940. (Biol. Abst. 16: 2926. 1942)

38. Bradford, F. C. and Joley, L. Infectious variegation of the apple. Jour. Agr. Res. 46: 901–908. 1933.

39. ———— and ————. Experiments in double working apple trees in the nursery. Proc. Am. Soc. Hort. Sci. 33: 360–365. 1936.

40. ———— and Sitton, G. B. Defective graft unions in the apple and the pear. Mich. Agr. Exp. Sta., Tech. Bull. 99. 1929.

41. Branas, M. Chlorose sur producteurs directs. Ann. École Nat. Agr. Montpellier 20: 58–62. (Biol. Abst. 8: 12981. 1934.)

42. Brase, K. D. Observations on Mazzard and Hahaleb seedlings bench-grafted to varieties of sweet and sour cherries. Proc. Am. Soc. Hort. Sci. 36: 110–112. 1939.

43. Brieger, F. G. and Gurgel, J. T. A. (The influence of rootstock on the pollen fertility of scion in citrus). Bragantia 1: 713–757. 1941. (Biol. Abst. 17: 6827. 1943.)

44. Brooks, F. T. and Brenchley, G. H. A note on the recovery from silver-leaf disease of plum trees on common plum and Myrobolan stocks, respectively. Jour. Pom. 13: 135–139. 1935.

45. Brown, G. G. Yields from young apple trees topworked in Arkansas. Proc. Am. Soc. Hort. Sci. 35: 141–142. 1938.

46. Brown, W. R. The orange—A trial of stocks at Peshawar. Agr. Res. Inst. of Pusa (India), Bull. 93: 1–6. 1920.

47. Bryant, L. R. Sour cherry rootstocks. Proc. Am. Soc. Hort. Sci. 36: 322–323. 1939.

48. Buder, J. Studien an *Laburnum adami*. II. Allgemeine anatomische Analyse den Mischlinge und seiner Stammpflanzen. Zeit. Ind. Abs. Ver. 5: 209–284. 1911.

49. Bunyard, E. A. Columella on graft hybrids. Gard. Chron. III. 49: 203. 1911.

50. Burchardt, H. (Grafting and budding cacao.) Tropenpflanzen 38: 239–245. 1935. (Hort. Abst. 5: 699.)

51. Cajlachjan, M. Ch. Motion of blossom hormone in girdled and grafted plants. Compt. Rend. (Doklady) Acad. Sci. U.R.S.S. 18: 607–612. 1938.

52. ————. (The effect of girdling and transplantation on the flowering of plants.) Izv. Akad. Nauk. S.S.S.R. Biol. Ser. 5–6. 1249–1279. 1938. (Herb. Abst. 9: 1571. 1939.)

53. ————. Translocation of flowering hormones in the plant as affected by temperature and narcotics. Compt. Rend. (Doklady) Acad. Sci. U.R.S.S. 31: 949–952. 1941.

54. ———— and Jarkovaja, L. M. (The effect of the stock on the flowering of the scion in *Perilla*.) Trudy Inst. Fiziol. Tumijazeu 2: 133–144. 1938. (Herb Abst. 8: 1351. 1938.)

55. Calvino, M. (A stock for dwarfing the olive tree.) Rep. IX Int. Hort. Congr. 252–254. 1930.

56. Camp, A. F. Citrus propagation. Fla. Agr. Exp. Sta., Bull. 227. 1931.

57. ————. The present status of rough lemon rootstock. Citrus Ind. 22: 5–7, 9, 18. 1941. (Biol. Abst. 15: 24228. 1941.)

58. Campbell, D. H. The nature of graft hybrids. Am. Nat. 45: 41–53. 1911.

59. Cardinell, H. A. and Bradford, F. C. Grafting in the apple orchard. Mich. Agr. Exp. Sta., Spec. Bull. 142. 1925.

60. Casella, D. (Grafting of olive on *Syringa vulgaris*.) Ann. R. Staz. Sperim. Frutticolt. e. Agrumicolt. Acircale N. S. 1: 211–214. 1934. (Biol. Abst. 10: 19257. 1936.)

61. Caspary, R. Ueber Pfropfbastarde. Comp. Rend. Congr. Amsterdam. 65–80. 1865.

62. Chang, W. T. Climatic influences on the affinity of pear grafts in Hangchow. Res. Pap. Nat. Univ. Chekiang. P. 16. 1933. (Hort. Abst. 5: 537. 1935.)

63. ————. Studies in incompatibility between stock and scion, with special reference to certain deciduous fruit trees. Jour. Pom. & Hort. Sci. 15: 267–325. 1938.

64. Chapin, W. S. Heredity in chimeras. Jour. Hered. 5: 533–546. 1914.

65. Chester, K. S. Graft-blight: a disease of lilac related to the employment of certain understocks in propagation. Jour. Am. Arb. 12: 79–146. 1931.

66. ————. Some notes on grafting. Canad. Hort. 55; 265. 1932.

67. ————. Studies on the precipitin reaction in plants. I. The specificity of the normal precipitin reaction. Jour. Arn. Arb. **13:** 52–74. 1932.

68. ————. Studies on the precipitin reaction in plants. II. Preliminary report on the nature of the "normal precipitin reaction". Jour. Arn. Arb. **13:** 285–296. 1932.

69. ———— and Whitaker, T. W. Studies on the precipitin reaction in plants. III. A biochemical analysis of the "normal precipitin reaction". Jour. Arn. Arb. **14:** 118–197. 1933.

70. ————. Studies on precipitin reaction in plants. IV. The question of acquired reactions due to grafting. Am. Jour. Bot. **20:** 297–308. 1933.

71. Cholodny, N. G. The internal factors of flowering. Herb. Rev. **7:** 223–247. 1939.

72. Clausen, R. E. and Goodspeed, T. H. Inheritance in *Nicotiana tabacum*. III. The occurrence of two natural periclinal chimeras. Genetics **8:** 97–105. 1923.

73. Clos, M. Quelques mots sur la Greffe. Rev. Hort. p. 342. 1856.

74. Colby, H. L. Stock-scion chemistry and the fruiting relationships in apple trees. Pl. Phys. **10:** 483–498. 1935.

75. Cooke, W. W. Plums and prunes on various rootstocks. Agr. Gaz. New South Wales **39:** 854–856. 1928.

76. Crafts, A. S. Phloem anatomy in two species of *Nicotiana,* with notes on the interspecific graft union. Bot. Gaz. **95:** 592–608. 1934.

77. Crane, M. B. *et al.* Studies on the resistance and immunity of apples to the wooly aphis. *Eriosoma Lanigerum* (Hansen). Jour. Pom. **14:** 137–163. 1936.

78. Cummings, M. B. and Jenkins, E. W. First 20 years of a variety apple orchard: apple cion selection. Vt. Agr. Exp. Sta., Bull. 255. 1926.

79. Daniel, L. Recherches morphologiques et philogiques sur la Greffe. Rev. Gén. Bot. **6:** 5–21; 60–75. 1894.

80. ————. Sur quelques applications pratiques de la greffe herbaceé. Rev. Gén. Bot. **6:** 356–369. 1894.

81. ————. La hybridation asexuelle. Rev. Gén. Bot. **26:** 305–341. 1914; **27:** 22–29; 33–49. 1915.

82. ————. Nouvelles recherches sur la migration de l'inuline dans les greffes de composeés. Compt. Rend. Acad. Sci. (Paris) **177:** 1135–1137. 1923.

82*a*. ————. Nouvelles observations sur les hybrides de greffe et hérédité chez les plantes greffes. Rev. Bret. Bot. **1924:** 1–70. 1925.

83. ————. Sur deux greffes nouvelles. Compt. Rend. Acad. Sci. (Paris) **184:** 1084–1085. 1927.

84. ————. Sur les variationes de la descendance des topinambours greffées. Compt. Rend. Acad. Sci. (Paris) **185:** 968–970. 1927.

85. ————. Variations de l'appareil sécréteur chez diverses plantes greffeés. Compt. Rend. Acad. Sci. (Paris) **185:** 1296–1298. 1927.

86. ————. Nouvelles observations sur les variations de la descendance de topinambours greffeés. Compt. Rend. Acad. Sci. (Paris) **186:** 785–787. 1928.

87. ————. Sur les variations de l'oxalate de calcium chez certaines plantes greffeés. Compt. Rend. Acad. Sci. (Paris) **186:** 1143–1144. 1928.

88. ————. Sur la formation des thylles chez les plantes greffeés. Compt. Rend. Acad. Sci. (Paris) **187:** 58–60. 1928.

89. ————. The inheritance of acquired characters in grafted plants. Proc. Int. Congr. Pl. Sci., Ithaca, N. Y. 1926. Vol. 2: 1024–1044. 1929

90. ————. Nouvelles recherches sur la descendance de certaines composées greffeés. Compt. Rend. Acad. Sci. (Paris) **194:** 1999–2002. 1932.
91. ————. Sur les curieuses variations des descendants de l'*Helianthus dangeardi* à la sixième génération. Compt. Rend. Acad. Sci. (Paris) **197:** 725–727. 1933.
92. ————. Variations des plantules d'*Helianthus dangeardi* à la septième génération sexuée. Compt. Rend. Acad. Sci. (Paris) **198:** 1962–1965. 1934.
93. ————. L'hérédité des monstruosités chez les descendants du topinambour greffé sur soleil annuel génération sexueé. Compt. Rend. Acad. Sci. (Paris) **201:** 801–803. 1935.
94. ————. Sur un cas indiscutable d'hérédité de caractère acquis. Compt. Rend. Acad. Sci. (Paris) **206:** 872–874. 1938.
95. Davis, L. D. and Tufts, W. P. Black-end of pears. III. Proc. Am. Soc. Hort. Sci. **33:** 304–315. 1935.
96. Davis, M. B. Possibility of the transmission by asexual propagation of the high yielding ability of individual apple trees. Sci. Agr. **2:** 120–124. 1921.
97. Dawson, R. F. The localization of the nicotine synthetic mechanism in the tobacco plant. Science **94:** 396–397. 1941.
98. ————. Accumulation of nicotine in reciprocal grafts of tomato and tobacco. Am. Jour. Bot. **29:** 66–71. 1942.
99. ————. Nicotine synthesis in excised tobacco roots. Am. Jour. Bot. **29:** 813–815. 1942.
100. ————. Accumulation of anabasine in reciprocal grafts of *Nicotiana glauca* and tomato. Am. Jour. Bot. **31:** 351–355. 1944.
101. ————. An experimental analysis of alkaloid production in *Nicotiana:* The origin of nornicotine. Am. Jour. Bot. **32:** 416–423. 1945.
102. Day, L. H. Ripening dates of Grand Duke plums on various understocks. Proc. Am. Soc. Hort. Sci. **30:** 357–360. 1933.
103. ————. The influence of seedlings of four peach varieties upon the growth of scions. Proc. Am. Soc. Hort. Sci. **35:** 383–385. 1938.
104. ———— and Tufts, W. P. Nematode-resistant rootstocks for deciduous fruit trees. Cal. Agr. Exp. Sta., Circ. 359. 1944.
105. Derickson, S. H. A natural graft between unrelated species. *Quercus rubra* and *Prunus avium*. Proc. Penn. Acad. Sci. **4:** 27–29. 1930.
106. Derman, H. Colchicine polyploidy and technique. Bot. Rev. **6:** 599–635. 1940.
106*a*. de Ropp, R. S. The growth-promoting and tumefacient factors of bacteria-free crown-gall tumor tissue. Am. Jour. Bot. **34:** 248–261. 1947.
107. Detjen, L. R. The influence of the rootstock on seeds and on seedling progenies of tomato grafts. Proc. Am. Soc. Hort. Sci. **43:** 147–148. 1943.
108. Eaton, F. M. and Blair, G. Y. Accumulation of boron by reciprocally grafted plants. Pl. Phys. **10:** 411–424. 1935.
109. Eigsti, O. J. A cytological study of colchicine effects in the induction of polyploidy of plants. Proc. Nat. Acad. Sci. **24:** 56–63. 1938.
110. Emon, J. Adaptation des porte-greffes aux différents terrains. Progr. Agr. Vitic. **106:** 521–524. 1936. (Hort. Abst. **7:** 48.)
111. Eseltine, G. P. van. Notes on the species of apples. I. The American crabapples. N. Y. (Geneva) Agr. Exp. Sta., Tech. Bull. 208. 1933.
112. Evenari, M. and Konis, E. The effect of hetero-auxin on root formation of cuttings and on grafting. Part I. Palestine Jour. Bot. (Jerusalem Ser.) **1:** 13–26. 1938. (Hort. Abst. **9:** 5.)
113. Featherby, H. G. Influence of stock on scion. Gard. Chron. **111:** 80. 1942.

114. Fikry, A. Nematode disease of stone fruits. Min. Agr. Egypt, Tech. & Sci. Serv., Bull. 217. 1939. (Biol. Abst. **14**: 3642. 1940.)

115. Filewicz, W. The frost injury of fruit trees in Poland in 1928–1929. Proc. IX Int. Hort. Congr. 312–320. 1930.

116. ————. (Reinvigoration of orchard trees by bridge grafting and inarching.) (Eng. sum.) (Reprint in Ann. Sci. Hort. **5**: 35–140. 1939.)

117. ———— and Modlibowaka, I. The influence of a scion variety on the resistance of the roots against frost. Proc. Am. Soc. Hort. Sci. **38**: 348–352. 1941.

118. Freeman, W. G. Vegetative propagation of cacao and West Indian citrus. Rep. IX Int. Hort. Cong. 348–357. 1930.

119. Friedrich, G. (Chemical and physiological changes in grafted apple trees). (*Reprint in* Kühn-Arch. **48**: 191–233. 1939). (Hort. Abst. **9**: 1158).

120. Fudge, B. R. Factors that influence fruit quality. Citrus Grower **2**: 3–4. 1940.

121. ———— and Fehmerling, G. B. Some effects of soils and fertilizers on fruit composition. Citrus Ind. **21**: 6, 7, 11, 14, 15. 1940.

122. Funck, R. (Untersuchungen über heteroplastische Transplantationen bei Solanacien und Cactaceen. Beitr. Biol. Pflanzen **17**: 404–468. 1929.

123. Gardner, F. E. and Lincoln, F. B. Factors influencing the variability of apple trees in the nursery row. Proc. Am. Soc. Hort. Sci. **29**: 367–370. 1932.

124. Garner, R. J. Double-working and bridging incompatible combinations of pear and quince. East Malling Res. Sta., Ann. Rep. **1943**. 80–85. 1944.

125. Gasis, D. Ueber die Unterscheidung verschiedener Pflanzeneiweissarten mit Hilfe spezifischer Sera. Berliner Klin. Wochenschr. **7**: 358–360. 1908.

126. Gladwin, F. E. Grafting American grapes on vigorous stocks. N. Y. (Geneva) Agr. Exp. Sta., Bull. 607. 1932.

127. Goldschmidt, R. Einfuehrung in die Vererbungswissenschaft. Ed. 3. 1920.

128. Goodale, S. L. Influence of the scion upon the stock. Horticulturist **1**: 290. 1846.

129. Goodspeed, T. H. and Uber, F. M. Radiation and plant cytogenetics. Bot. Rev. **5**: 1–48. 1939.

130. Gossard, A. C. Investigations on the congeniality of stock and scion. Master's Thesis, Univ. Wisconsin, 1929.

131. Green, F. The precipitin reaction in relation to grafting. Genetics **11**: 73–82. 1926.

132. Greenleaf, W. H. Induction of polyploidy in *Nicotiana* by heteroauxin treatment. Jour. Hered. **29**: 451–464. 1938.

133. Griffen, E. Greffage et hybridation asexuelle. Conf. Int. Genet., Paris. **1911**: 164–196. 1913.

134. Grubb, N. H. Experiments with double-worked pears on quince stocks. East Malling Res. Sta., Ann. Rep. **1927**, II Suppl. 11–15.

135. ————. Cherry stocks at East Malling. I. Stocks for Morello cherries. Jour. Pom. & Hort. Sci. **11**: 276–304. 1933.

136. ————. Influence of the intermediate in double-worked apple trees; nursery trials of the "stem-builder" process at East Malling. Jour. Pom. & Hort. Sci. **17**: 1–19. 1939.

137. Haas, A. R. C. and Halina, F. F. Chemical relationship between scion and stock in citrus. Pl. Phys. **4**: 113–121. 1929.

138. Haberlandt, G. Ueber den Blattbau der Crataego-mespili von Bronvaux und ihrer Eltern. Sitzungber. Preuss. Akad. Wiss. **17**: 170–208. 1926. (Biol. Abst. **1**: 8565. 1927).

139. Hafekost, G. Über die Beziehungen zwischen Edelreis und Unterlage bei einigen Obstarten. Gartenbauwiss. **7:** 382–395. 1933.
140. Hall, E. G. Effects of stock on citrus fruit quality. Agr. Gaz. New South Wales **54:** 173–176. 1943. (Biol. Abst. **18:** 5262. 1944).
141. Halma, F. F. An attempt to recover declined lemon trees by use of inarching. Cal. Citrograph. **17:** 349, 365–367. 1932.
142. ————. Scion influence in citrus. Jour. Pom. & Hort. Sci. **12:** 99–104. 1934.
143. Hamner, K. C. and Bonner, J. Photoperiodism in relation to hormones as factors in floral initiation and development. Bot. Gaz. **100:** 388–431. 1938.
144. Harris, R. V. Grafting as a method for investigating a possible virus disease of the strawberry. Jour. Pom. & Hort. Sci. **10:** 35–41. 1932.
145. ———— and Wormald, H. Diseases of tree fruits. East Malling Res. Sta., Ann. Rep. **1940:** 23–24.
146. Hatton, R. G. Paradise apple stocks: Their fruit and blossom described. Jour. Royal Hort. Soc. **44:** 89–94. 1919.
147. ————. Apple root-stocks, their particular suitabilities for different soils, varieties and purposes. East Malling Res. Station, Ann. Rep. **1925:** 45–52.
148. ————. The behavior of certain pears on various quince rootstocks. Jour. Pom. & Hort. Sci. **7:** 216–233. 1928.
149. ————. The development of a research programme around the "build-up" of a fruit plant. Proc. Royal Hort. Soc. 245–251. London 1930.
150. ————. Rootstocks for pears. East Malling Res. Sta., Ann. Rep. **1935:** 75–86.
151. ————. Plum rootstock studies: Their effect on the vigor and cropping of the scion variety. Jour. Pom. & Hort. Sci. **14:** 97–136. 1936.
152. ———— and Grubb, N. H. Some factors influencing the period of blossoming of apples and plums. Jour. Pom. & Hort. Sci. **5:** 210–215. 1926.
153. ————, Amos, J. and Witt, A. W. Plum rootstocks; their varieties, propagation and influence upon cultivated varieties worked thereon. Jour. Pom. & Hort. Sci. **7:** 64–99. 1928.
154. ———— *et al.* The effect of scion on root. II. Stem-worked apples. Jour. Pom. & Hort. Sci. **8:** 248–258. 1930.
155. Hayward, H. E. and Long, E. M. Vegetative responses of the Elberta peach and Lovell and Shalil root-stocks to high chloride and sulfate solutions. Proc. Am. Soc. Hort. Sci. **41:** 149–155. 1942.
156. ———— and Went, F. W. Transplantation experiments with peas. II. Bot. Gaz. **100:** 788–801. 1939.
157. Hearman, J. (Miss). The Northern Spy as a rootstock when compared with other standardized European rootstocks. Jour. Pom. & Hort. Sci. **14:** 246–275. 1936.
158. ———— *et al.* The reinvigoration of apple trees by the inarching of vigorous rootstocks. Jour. Pom. **14:** 376–390. 1936.
159. Hendrick, U. P. Stocks for plums. N. Y. (Geneva) Agr. Exp. Sta., Bull. 498. 1923.
160. Heinicke, A. J. Influence of scion leaves on the quality of apples borne by the stock. Proc. Am. Soc. Hort. Sci. **24:** 143–146. 1927.
161. ————. To what extent is the McIntosh apple influenced by the food materials synthesized by Northern Spy leaves. Proc. Am. Soc. Hort. Sci. **33:** 336–337. 1935.
162. Heinze, P. H. *et al.* Floral initiation in Biloxi soybean as influenced by grafting. Bot. Gaz. **103:** 518–530. 1942.
163. Heppner, M. J. Pear black end and its relation to different rootstocks. Proc. Am. Soc. Hort. Sci. **24:** 139–142. 1928.

164. ———— and McCallum, R. D. Grafting affinities with special reference to plums. Cal. Agr. Exp. Sta., Bull. 438. 1927.

165. ———— and ————. Compatible and non-compatible graft unions. Proc. Am. Soc. Hort. Sci. **24**: 137–138. 1928.

166. Herbert, D. A. Nutritional exchange between lianas and trees. Proc. Royal Sci. Soc. Queensland **39**: 115–118. 1927. (Biol. Abst. **6**: 18443. 1932).

167. Hewetson, F. N. Growth and yield of McIntosh apple trees as influenced by the use of various intermediate stem pieces. Proc. Am. Soc. Hort. Sci. **45**: 181–186. 1944.

168. Hieke, K. Pflanzenphysiologische Untersuchungen über die Alkaloide. II. Zur Alkaloidführunden Pfropfpartner bei heteroplastischen Solanaceen Pfropfungen. Planta **33**: 185–205. 1942.

169. Heuer, H. Ueber Pfropftbastarde zwischen *Solanum* Arten. Gartenflora **59**: 434–438. 1910.

170. Hiesey, W. M. Environmental influence and transplant experiments. Bot. Rev. **6**: 181–203. 1940.

171. Hilborn, M. T. and Waring, J. H. Terminal-shoot growth of apple varieties as apparently stimulated by Virginia Crab and Hibernal intermediate stocks. Proc. Am. Soc. Hort. Sci. **38**: 316–320. 1941.

172. Himmelbaur, W. Der gegenwaertige Stand der Pfropfhybridenfrage. Mitt. Naturw. Ver. Univ. Wien. **8**: 105–127. 1910.

173. Hirche, R. Pfropfhybriden bei Kartoffeln. Mitt. Deut. Landw. Ges. **24**: 442–444. 1909.

174. Hoblyn, T. N. Manurial trials with apple trees at East Malling 1920–39. Jour. Pom. & Hort. Sci. **18**: 325–343. 1941.

175. Hockey, J. F. Mosaic, false sting and flat limb of apple. Sci. Agr. **23**: 633–646. 1943.

176. Hodgson, R. W. Rootstocks for the oriental persimmon. Cal. Avocado Assoc., Yearbook **1940**. 43–44. 1940. (Biol. Abst. **15**: 24258. 1941).

177. ———— and Eggers. E. R. Rootstock influence on the composition of citrus fruits. Cal. Citrograph. **23**: 499, 531. 1938. (Biol. Abst. **13**: 1244. 1939).

178. Hofmann, F. W. Reciprocal effects from grafting. Jour. Agr. Res. **34**: 673–676. 1927.

179. Hoop, D. J. N. van der and Ostendorf, F. W. (Mutual influence of stock and scion in *Hevea* buddings). Arch. Rubbercult. Nederlandsch. Indie **16**: 392–409. 1932. (Biol. Abst. **8**: 14166. 1934).

180. Hoopes, J. Influence of the stock on the scion, and vice versa. Hort. **28**: 355. 1873.

181. Hoser, P. (A graft-hybrid of lilacs). (Ann. Sci. Hort. [Warsaw]). **1**: 111–120. 1934. (Biol. Abst. **10**: 2617. 1936).

182. Howard, W. L. and Heppner, M. J. Graft affinity tests with peach on Myrobolan and Mariana plums. Proc. Am. Soc. Hort. Sci. **25**: 178–180. 1929.

183. Howe, G. H. Relative merits of Mazzard and Mahaleb root-stocks for cherries. Proc. Am. Soc. Hort. Sci. **23**: 53–55. 1927.

184. Hubbell, D. S. Causes of blind wood in roses. Pl. Phys. **9**: 261–282. 1934.

185. Hume, M. On the presence of connecting threads in graft hybrids. New Phyt. **12**: 216–221. 1913.

186. Husmann, G. C. Grape investigations in the Vinifera regions of the United States with reference to resistant stocks, direct producers, and Viniferas. U. S. Dept. Agr., Bull. 172. 1910.

187. ————. Testing phyloxera-resistant grape stocks in the Vinifera regions of the United States. U. S. Dept. Agr., Tech. Bull. 146. 1930.

188. Hutchins, L. M. Nematode-resistant peach root stock of superior vigor. Proc. Am. Soc. Hort. Sci. **34**: 330–338. 1937.

189. Jacobs, H. E. Examples of incompatibility between grape varieties and rootstocks. Proc. Am. Soc. Hort. Sci. **41**: 201–203. 1942.

190. Jensen, C. A. *et al.* Behavior of different lemon bud and rootstock combinations at Limoneira. Cal. Citrograph. **12**: 234, 266–268. 1927.

191. Johnston, S. Bridge-grafting the peach. Mich. Agr. Exp. Sta., Quart. Bull. **19**: 177–179. 1937.

192. ————. *Prunus Mexicana* and *Prunus hortulana* as rootstocks for peaches Mich. Agr. Exp. Sta., Quart. Bull. **21**: 17–18. 1938.

193. Joley, L. E. Overgrowth produced by edible cherry on Sargent cherry (*Prunus sargentii* Rebd.). Proc. Am. Soc. Hort. Sci. **40**: 277–278. 1942.

194. Jones, M. A. and Cooper, W. C. The lack of scion effect on root quality of *Derris elliptica*. Pl. Phys. **21**: 63–67. 1946.

195. Jones, W. W. Plant chimeras and graft hybrids. 1934.

196. ————. Chimeras: a summary and some special aspects. Bot. Rev. **3**: 545–562. 1937.

197. Jörgensen, C. A. The experimental formation of heteroploid plants in the genus *Solanum*. Jour. Genet. **19**: 133–211. 1927–8.

198. ———— and Crane, M. B. Formation and morphology of *Solanum* chimeras. Jour. Genet. **18**: 247–273. 1927.

199. Kác. V. (Grafting of fruit plants). (Biol. Abst. **8**: 11819. 1934).

200. Keitt, G. W. and Clayton, C. N. A destructive bud-transmissible disease of sour cherry in Wisconsin. Phytopath. **29**: 821–822. 1939.

201. Kemmer, E. Stand der Unterlagenforschung im Obstbau. Forschungsdienst **1**: 268–284. 1936. (Hort. Abst. **6**: 265).

202. Kench, J. E. The seasonal cycles of ash, carbohydrate and nitrogenous constituents in the terminal shoots of apple trees and the effects of five vegetatively propagated rootstocks on them. III. Nitrogenous constituents. Jour. Pom. **16**: 346–363. 1939.

203. Kerr, W. L. A simple method of obtaining fruit trees on their own roots. Proc. Am. Soc. Hort. Sci. **33**: 355–357. 1936.

204. Khosina, E. P. (Mme.) The change in the shape of fruit in the seed progeny of grafts. Jarovizatsiia **1**: 84–89. 1941. (Biol. Abst. **16**: 3573. 1942).

205. Knight, R. C. Preliminary observation on the causes of stock influences in apples. East Malling Res. Sta., Ann. Rep. **1927. 13 II** Suppl.: 51–63.

206. ————. A note on the orientation of main branches and its relation to stock in some "double stock" apple trees. East Malling Res. Sta., Ann. Rep. 1926/1927. I Suppl.: 11–30. 1928.

207. ————. Further observations on the parts played by root and stem in stock influence. East Malling Res. Sta., Ann. Rep. **1934**: 114–116.

208. Kordes, H. Bedeutung der Wuchsstoffe für die vegetative Vermehrung der Rebe, insbesondere für die Rebenvermehrung. Ang. Bot. **19**: 543–544. 1937. (Hort. Abst. **8**: 62, 431)

209. Korshunov, K. N. (Sorbus as an understock for pears). (Biol. Abst. **18**: 1567. 1944)

210. Kostoff, D. Induced immunity in plants. Nat. Acad. Sci. **14**: 236–237. 1928.

211. ————. Studies on callus tissue. Am. Jour. Bot. **15**: 565–576. 1928.

212. ————. Acquired immunity in plants. Genetics **14**: 37–77; 1929.

213. ————. Chromosomal aberrants and gene mutations in *Nicotiana* obtained by grafting. Jour. Genet. **22**: 399–418. 1930.

214. ————. (Biology of the callus). Univ. Sofia, Fac. Agron., Yearbook **8**: 297–316. 1929/30. (Biol. Abst. **6**: 4877. 1932)

215. ————. (Studies on acquired immunity in plants. A contribution to methods of investigation of reciprocal activities between graft components.) Svedonia po Zemledelieto **12**: 47–68. 1931. (Biol. Abst. **7**: 354. 1933)

216. ————. (Sterility, transplantation and immunity). Zemledelska Misal **1**: 5–38. 1931. (Biol. Abst. **7**: 341. 1933)

217. Kovalevskaya, P. J. (Grafting as a condition of form-forming in the sex hybrid of tomatoes.) Jarovisatsiia **1**: 118–121. 1939.

218. Krajevoj, S. J. and Nechaev, I. Atropine transference from stock (*Datura stramonium*) to scion (*Solanum lycopersicum*). Compt. Rend. (Doklady) Acad. Sci. U.R.S.S. **31**: 36–71. 1941.

219. Krenke, N. P. Wundkompensation, Transplantation und Chimären bei Pflanzen. *In* Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Tiere, Vol. 29. [Transl. from Russian by Busch, N.] 934 pp. 1933. (Biol. Abst. **8**: 17457. 1934)

220. Kuijper, J. and Wiersum, L. K. Occurrence and transport of a substance causing flowering in the Soya bean (*Glycine Max L.*) Proc. Royal Acad. Sci., Amsterdam. **39**: 1114–1122. 1936.

221. Kunkel, L. O. Studies on aster yellows in some new host plants. Contr. Boyce Thompson Inst. **3**: 85–123. 1931.

222. ————. Contact periods in graft transmission of peach viruses. Phytopath. **28**: 491–497. 1938.

223. Lagasse, F. S. A comparison of the top and root growth made by five varieties of apples on seedlings and scion roots during five years in the orchard. Proc. Am. Soc. Hort. Sci. **33**: 366–371. 1936.

224. Lange, F. Vergleichende Untersuchungen über die Blattentwicklung einiger Solanum—Chimären und ihrer Elterarten. Planta **3**: 181–281. 1927. (Biol. Abst. **2**: 13364. 1928)

225. Largillier, H. J. Étude sur le greffage. Prog. Agr. Vit. **106**: 204–206. 1936. (Hort. Abst. **6**: 460.)

226. Laurent, C. Sur les variations de composition de certaines plantes alimentaires après greffage Trans. Sci. Univ. Rennes **5**: 141–147. 1906.

227. Lawson, W. A new orchard and garden. 1660.

228. Lebedeva, S. P. Changing the nature of plants (melons) by grafting [Russian]. Nov. Sel. Khoz, Moscow No. 16: 1–12. 1937. (Herb. Abst. **8**: 1827. 1938)

229. Lincoln, F. B. Some apple tree stock relationships seen in New England after 1938 hurricane. Proc. Am. Soc. Hort. Sci. **36**: 102–109. 1938.

230. ————. Scion influence on nursery apple tree roots. Proc. Am. Soc. Hort. Sci. **39**: 237–239. 1941.

231. ————. Evidence of scion influence on stock. Proc. Am. Soc. Hort. Sci. **39**: 240–244. 1941.

232. Lindemuth, H. Vegetative Bastarderzeugung durch Impfung (inoculation). Landw. Jahrb.**7**: 887–939. 1878.

233. ————. Das Verhalten durch Copulation verbundener Pflanzenarten. Ber. Deut. Bot. Ges. **19**: 515–529. 1901.

234. ————. Über angebliches Vorhandensein von Atropin in Kartoffelknollen infolge von Transplantation und über die Grenzen der Verwachsung nach dem Verwandschaftsgrade. Ber. Deut. Bot. Ges. **24**: 428–435. 1906.

235. Long, E. M. The effect of salt additions to the substrate on intake of water and nutrients by roots of approach-grafted tomato plants. Am. Jour. Bot. **30**: 594–601. 1943.

236. Loomis, N. H. *et al.* Further studies on the adaptability of some American grape varieties to southern conditions when grown on their own roots and on certain stocks. Proc. Am. Soc. Hort. Sci. **42**: 391–394. 1943.
237. Lugeon, A. R. Top-grafting cherries and plums. Gard. Chron. **97**: 98. 1935.
238. Lynch, S. J. Some analytical studies of the Persian lime. Fla. Agr. Exp. Sta., Bul. 368. 1942.
239. MacCallum, W. B. The reciprocal influence of scion and stock. Pl. World **12**: 281–286. 1909.
240. MacDaniels, L. H. Tree bracing and wound treatment in the orchard. N. Y. (Cornell) Agr. Exp. Sta., Bull. 312. 1935.
241. Magoon, C. A. *et al.* Investigations on the adaptability of grape root stocks to Gulf Coast conditions. Proc. Am. Soc. Hort. Sci. **35**: 466–470. 1937.
242. ———— *et al.* A study of rootstocks of Concord, Ontario, and Delaware grapes. Proc. Am. Soc. Hort. Sci. **46**: 222–226. 1945.
243. Maney, T. J. Stock and scion relationships with reference to double worked apple stocks. Proc. Am. Soc. Hort. Sci. **35**: 390–392. 1938.
244. ————. The growth and production of topworked apple varieties. Proc. Am. Soc. Hort. Sci. **36**: 287–290. 1939.
245. ————. Records on a full crop yield of apple varieties topworked on various hardy stocks. Proc. Am. Soc. Hort. Sci. **38**: 345–347. 1941.
246. Margar'yan, A. E. Vegetative propagation of the tung tree [Russian; Eng. sum.]. Soviet Subtropics No. *2*(42): 10–21. 1938. (Hort. Abst. **8**: 822)
247. Marloth, R. H. The citrus rootstock problem. Citrus-tree propagation. Farming in So. Africa. **13**: 226–231. 1938. (Biol Abst. **14**: 7461. 1940)
248. Marshall, T. H. Coffee grafting and budding. Trop. Agr. **84**: 93–96. 1935. (Hort. Abst. **5**: 285)
249. Martin, J. N. The development of new lateral roots in relation to the crown buds in *Melilotus alba.* Proc. Iowa Acad. Sci. **40**: 79. 1933.
250. Mattras, H. Expérience de surgreffage de la vigne dans le Gard. Prog. Agr. Vit. **105**: 200–202. 1936. (Hort. Abst. **7**: 47)
251. Matubara, S. (Experiments on the grafting of fruit trees and their anatomical and physiological observations in relation to its success). (Eng. sum.). (Bul. Miyazaki Coll. Agr. & For. **3**: 21–42. 1931). (Biol. Abst. **6**: 20015. 1932)
252. McClintock, J. A. Peach rosette, an infectious mosaic. Jour. Agr. Res. **24**: 307–315. 1923.
253. ————. Further evidence of uncongeniality in disease resistant stocks. Proc. Am. Soc. Hort. Sci. **22**: 231–232. 1926.
254. ————. American Mazzard cherry seedlings as rootstocks for cultivated cherries. Proc. Am. Soc. Hort. Sci. **26**: 82–85. 1930
255. ————. The affinity of varieties other than Grimes on Virginia Crab stocks. Proc. Am. Soc. Hort. Sci. **35**: 131–132. 1938.
256. ————. The effect of stocks on the yield of Grimes apples. Proc. Am. Soc. Hort. Sci. **35**: 369–371. 1938.
257. ————. Preliminary studies on adapting Virginia Crab to topworking with Stayman. Prop. Am. Soc. Hort. Sci. **45**: 177–180. 1944.
258. Melchers, G. Die Blühhormone. Ber. Deut. Bot. Ges. **57**: 29–48. 1939. (Herb. Abst. **9**: 1140. 1939)
259. Mendel, K. The anatomy and histology of the bud-union in citrus. Palestine Jour. Bot. & Hort. Sci. **1**: 3–46. 1936.

260. Meyer, A. and Schmidt, E. Über die gegenseitige Beeinflussung der Symbionten heteroplastischer Transplantionen mit besonderer Berücksichtigung der Wanderung der Alkaloide durch die Pfropfstellen. Flora **100**: 317–397. 1910.

261. Milbrath, J. A. and Zeller, S. M. Latent viruses in stone fruits. Science **101**: 114–115. 1945.

262. Millner, M. E. Natural grafting in *Hedera helix*. New Phyt. **31**: 2–25. 1932.

263. Mirov, N. T. Tested methods of grafting pines. Jour. For. **38**: 768–777. 1940.

264. Mitchell, H. L. The development of a high-yielding strain of naval stores pine to increase the output of oleoresin per tree. Naval Stores Rev. **52**: 10, 12. 1942. (Biol. Abst. **17**: 17215. 1943)

265. Molotkovsky, G. Kh. Some results in changing the nature of plants by means of grafting. Compt. Rend. (Doklady) Acad. Sci. U.R.S.S. **24**: 602–604. 1939.

267. Monakima, T. A. and Solovey, G. T. Grafting monocotyledonous bulbous plants. [Russian]. Vernalization No. 4 140–141. 1939. (Hort. Abst. **10**: 475).

268. Moore, M. H. Spraying experiments on the control of pear scab at East Malling. Jour Min. Agr. (Gr. Brit) **40**: 111-119 1933. (Biol. Abst **9**: 11069. 1935)

269. Moreira, S. (An interesting case of incongeniality in citrus grafts). Jour. Agron. **1**: 57–64. 1938. (Biol. Abst. **12**: 16676 1938)

270. Moshkov, B. S. (Photoperiodic response of leaves and its utilization in grafting). Trudy Prikl. Bot. Genet. i. Selekcii. Ser. A. No. 19. 107–126. 1936. (Herb. Abst. **7**: 326. 1937)

271. ————. (Flowering of short-day plants under continuous day as a result of grafting). Trudy Prikl. Bot. Genet i Selekcii Ser. A No. 21. 145–156. 1937. (Herb. Abst. **8**: 634. 1938)

272. ————. Blooming of short-day plants in a condition of continuous illumination as a result of grafting. Bull. Appl. Bot., Genet. & Pl. Breed., A., Suppl. **21**: 145–146. 1937.

273. ————. Symbiosis of leguminous plants with nitrogen assimilating bacteria as determined by photoperiodism. Compt. Rend. (Doklady) Acad. Sci. U.R.S.S. **22**: 187–188. 1939.

274. ———— and Smirnova, M. I Grafting as a method of changing alkaloid content in plants. Compt. Rend. (Doklady) Acad. Sci. U.R.S.S. **24**: 88–90. 1939.

275. ————. Transfer of photoperiodic reaction from leaves to growing points. Compt. Rend. (Doklady) Acad. Sci. U.R.S.S. **24**: 489–491. 1939.

276. Mottier, D. M. Plant chimeras and their relation to hereditary phenomena. Sch Sci. & Math. **15**: 713–716. 1915.

277. Müller-Stoll, W. R. Weitere Versuche über die Verwendbarkeit von Wuchsstoffen in der Rebenveredlung. Gartenbauwiss. **14**: 151–168. 1940. (Biol. Abst. **15**: 15537. 1941)

278. Namikawa, I. (On the chromosomes of the persimmon). Tottor Nogaku-Kwailo (Tottori Soc. Agr. Sci.) **2**: 145–148. 1930 (Biol. Abst. **8**: 105. 1934)

279. Nawaschin, M. Alten der Samen als Ursache von Chromosomenmutationen. Planta **20**: 233–243. 1933.

280. Nebel, B. R. and Ruttle, M. L. The cytological and genetical significance of colchicine. Jour. Hered. **29**: 3–9. 1938.

281. Neiman, N. F. (On the formation of irregular thickenings of the cell walls in graft unions of beet). Bull. Appl. Bot., Genet. & Pl. Breed., A. Pl. Ind., U.S.S.R. **5/6**: 147–151. 1933.

282. Neuwirth, Fr. Xr. (An interesting graft). Mykologia (Prague) **3:** 51. 1926. (Biol. Abst. **2:** 18673. 1928)

283. Nolle, F. Pfropf- und Verwachsungsversuche mit Siphoneen. Sitz. Niederr. Ges. Nat. u. Heil. 1897.

284. Obsil, K. Zur Frage der Blühhormone. Planta **29**: 468–476. 1939. (Biol. Abst. **13:** 15, 713. 1939)

285. Okanenko, A. S. and Vandivk, N. V. Physiological basis of mutual influence between stock and scion of beet. Compt. Rend. (Doklady) Acad. Sci. U.R.S.S. **24:** 807–811. 1939.

286. Ohmann, M. Ueber die Art und der Zustandekommen des Wachsung zweier Pfropfsymbionten. Centralbl. Bakt. Abt. 2. **21:** 232–256; 318–329. 1908.

287. Ostendorf, F. W. (The growth of young *Hevea* buddings). 1933. (Biol. Abst. **8:** 14175. 1934)

288. Overholtzer, E. L. *et al.* Nursery fruit trees, dwarf and standard understocks, their handling and planting. Wash. Agr. Exp. Sta., Pop. Bull. 170. 1943.

289. Palmer, R. C. and Hewetson, F. N. Stock and scion relationships in some four year old apple trees. Sci. Agr. **17:** 551–562. 1937.

290. Pasinetti, L. (The death of grafted vinifera vines). Riv. Patol. Veg. **28:** 81–101. 1938. (Biol. Abst. **12:** 7512. 1938)

291. Paul, W. R. C. and Guneratuam, S. C. The propagation of the mango in Jaffua. Trop. Agriculturist **88:** 331–337. 1937. (Hort. Abst. **8:** 248)

292. Paulsen, F. and Berna, R. (Second note on the "Ciminnita" grape. Artificial pollination). Italia Agr. **71:** 117–9. 1934. (Biol. Abst. **11:** 6818. 1937.)

293. Pavskii, V. A. (Concerning apple understocks in the prikamsk portion of the Molotovsk province). (Biol. Abst. **18:** 7160. 1944.)

294. Peacock, S. M. *et al.* Alkaloid accumulation in reciprocal grafts of *Datura Stramonium* with tobacco and tomato. Am. Jour. Bot. **31:** 463–466. 1944.

295. Pearse, H. L. Water-culture studies with apple trees. II. The seasonal absorption of nitrogen and potassium by Cox's Orange Pippin on Malling rootstocks. Nos. IX and XII. Jour. Pom. **17:** 344–361. 1940.

296. Peto, F. H. Genetical studies on mutants in the progeny of heat-treated barley. Canad. Jour. Res. **15:** 217–229. 1937.

297. Pirschle, K. Über den Mineralsroffwechsel von homo- und heteroplastischen Pfropfungen mit Petunia D und d. Biol. Zentralbl. **59:** 123–157. 1939. (Biol. Abst. **13:** 10315. 1939)

298. Plotnikov, I. G. (Technique of grafting of grasses). Jarovizacija No. 3 63–65. 1939. (Herb. Abst. **10:** 619. 1940)

299. ————. (Grafting of graminaceous plants). Soc. Zern. Hoz. No. 4: 109–117. 1939. (Herb. Abst. **10:** 775. 1940).

300. Polishtshonk, A. D. (Influence of the original position of the scion used for grafting on the development of grafted apples). Doklady Usesoinznoi Akademii S.-Kh. Nauk. **13:** 14–18. 1940. (Bot. Abst. **16:** 16935. 1942)

301. Pope, W. T. and Story, W. B. Grafting tropical fruit trees in Hawaii. Hawaii Agr. Exp. Sta., Circ. 6: 1933.

302. Popeso, C. T. Effets d'un nouveau système de greffage chez le *Lycium vulgare* placé sur la tomate. Compt. Rend. Acad. Sci. (Paris) 1943: 1129–1131. 1932. (Biol. Abst. **8:** 11834. 1934)

303. Pound, F. J. The significances of budding and grafting cacao and of producing rooted cuttings. Proc. Agr. Soc. Trinidad & Tobago. **43:** 65–74. 1943. (Biol. Abst. **17:** 513. 1944)

304. Proebsting, E. L. Structural weakness in interspecific grafts of *Pyrus*. Bot. Gaz. **82**: 336–338. 1926.
305. ———— and Barger, E. H. The precipitin reaction as a means of determining the congeniality of grafts. Science **65**: 573–574. 1927.
306. ————. Further observations on structural defects of the graft union. Bot. Gaz. **86**: 82–92. 1928.
307. ———— and Hansen, C. J. Leaf scorch and die-back of apricots. Proc. Am. Soc. Hort. Sci. **42**: 270–274. 1943.
308. Provan, J. L. and Greatorex, F. J. Invigorating fruit trees by inarching. Jour. Dept. Agr. Victoria **38**: 366–368. 1940. (Biol. Abst. **16**: 5274. 1942)
309. Quinn, G. The influence of rootstocks on the texture and flavor of orange fruits. So. Australia Dept. Agr., Bull. 276. 1933.
310. ————. Rootstocks for cherry trees. Jour. Dept. Agr. So. Australia **38**: 1109–1124; 1245–1262. 1935.
311. ————. Rootstocks for peach trees. Jour. Dept. Agr. So. Australia **38**: 1509–1539. 1935.
312. Raleigh, W. D. An abnormal graft reaction in potato resulting from a virus infection of a scion on a resistant stock. Phytopath. **26**: 795. 1936.
313. Rao, Y. V. and Berry, W. E. The carbohydrate relations of a single scion variety grafted on Malling rootstocks IX and XIII; a contribution to the physiology of dwarfing. Jour. Pom. & Hort. Sci. **18**: 193–225. 1940.
314. Ravaz, L. De l'influence spécifique réciproque du sujet et du greffon. Rep. IX Int. Hort. Congr. 255–263. 1930.
315. Rawlins, T. E. and Home, W. T. "Buckskin", a destructive graft-infectious disease of the cherry. Phytopath **21**: 331–335. 1931.
316. ———— and Parker, K. G. Influence of rootstock on susceptibility of sweet cherry to buckskin disease. Phytopath. **24**: 1029–1031. 1934.
317. Razumov, V. I. (Behavior of individual plants of a pure line under varying day length). Jarovizacija No. **2**: 74–82. 1937. (Herb. Abst. **8**: 215. 1938).
318. ————. (The change of hereditary characteristics of potato by grafting). (Biol. Abst. **14**: 15693. 1940).
319. Reed, C. A. Nut-tree propagation. U. S. Dept. Agr., Farm. Bull. 1501. 1926.
320. ————. The present status of chestnut growing in the United States. Proc. Am. Soc. Hort. Sci. **39**: 147–152. 1941.
321. Reeves, E. L. Mottle-Leaf, a virus disease of cherries. Jour. Agr. Res. **63**: 555–572. 1941.
322. Reimer, F. C. Blight resistance in pears and characteristics of pear species and stocks. Ore. Agr. Exp. Sta., Bull. 214. 1925.
323. Reynolds, H. and Vaile, J. E. Effects of rootstock upon composition and quality of fruit of Concord, Campbell Early and Moore Early grapes. Ark. Agr. Exp. Sta., Bul. 421. 1942.
324. Richards, A. V. Studies on stock-scion interaction in citrus. I. Growth and development of seedling stocks and young grafts. Trop. Agr. (Ceylon) **91**: 12–24. 1938.
325. ————. Influence of rootstock on quality of citrus fruit. Trop. Agr. (Ceylon) **94**: 354–361. 1940. (Biol. Abst. **16**: 5286. 1942).
326. ————. Studies on propagation of seedless Tahiti lime. Trop. Agr. (Ceylon) **98**: 9–12. 1942. (Biol. Abst. **18**: 14397. 1944).
327. ————. Studies in propagation of Sapodilla. Trop. Agr. (Ceylon) **99**: 78–82. 1943. (Biol. Abst. **18**: 14398. 1943).
328. Richmond, T. E. Legume inoculation as influenced by stock and scion. Bot. Gaz. **82**: 438–442. 1926.

329. RICHTER, O. Pfropfungen, Pfropfbastarde, und Pflanzenchimaeren. Lotos **508**: 1–22; 39–51. 1910.

330. RIKER, A. J. *et al.* Antiseptic solutions and antiseptic adhesive tape in relation to control of hairy root, crown gall, and other growths on nursery apple trees. Phytopath. **25**: 192–207. 1935.

331. RIVES, L. Sur l'emploi du séro-diagnostic pour la détermination de l'affinité au greffage des hybrides de vigne. Prog. Agr. et Vit. **79**: 118–119. 1923.

332. ————. Nouvelle contribution à l'étude de l'affinité. Rev. Vit. **71**: 397–405. 1929. (Biol. Abst. **5**: 27160. 1931).

333. ROACH, W. A. Increased scion vigour induced by certain foreign rootstocks. Ann. Bot. **44**: 859–864. 1930. (Biol. Abst. **7**: 5276. 1933).

334. ————. The chemistry of the rootstock-scion effect. I. The elements absorbed from the soil. East Malling Res. Sta., Ann. Rep. **1928–1930**. II Suppl. 101–104. 1931.

335. ———— and BOLAS, B. D. Review of research. IV. Biochemistry. East. Malling Res. Sta., Ann. Rep. **1944**. 24–25.

336. ROBERTS, R. H. Variation in growth of nursery grafts. Science **62**: 356. 1925.

337. ————. Factors affecting the variable growth of apple grafts in the nursery row. Wis. Agr. Exp. Sta., Res. Bull. 77: 1927.

338. ————. Some stock and scion observations on apple trees. Wis. Agr. Exp. Sta., Res. Bull. 94. 1929.

339. ————. Two more season's notes upon stock and scion relation. Proc. Am. Soc. Hort. Sci. **27**: 102–105. 1930.

340. ————. Notes upon stock and scion relations in 1931. Proc. Am. Soc. Hort. Sci. **28**: 470–472. 1931.

341. ————. Ring grafting and stock effect. Proc. Am. Soc. Hort. Sci. **32**: 328–329. 1934.

342. ————. A further trial of ring grafting to produce stock effects. Proc. Am. Soc. Hort. Sci. **33**: 358–359. 1935.

343. ————. A third experience in producing stock effects with ring grafts. Proc. Am. Soc. Hort. Sci. **34**: 296–297. 1936.

344. ————. Top and double-working trees. Wis. Agr. Exp. Sta., Bull. 432. 1936.

345. ———— and STRUCKMEYER, B. ESTHER. The point of origin of the blossom-inducing stimulus. Science **90**: 16. 1939.

346. ROGERS, W. S. Rootstock effect on colour and size of apples. East Malling Res. Sta., Ann. Rep. 13: **1927**. 16–32.

347. ———— and VYVYAN, M. C. The root systems of some ten-year-old apple trees on two different rootstocks, and their relation to tree performance. East Malling Res. Sta., Ann. Rep. **1928**. II Suppl. 31–43.

348. ———— *et al.* The influence of "stem-builder" intermediates on apple root systems. Jour. Pom. & Hort. Sci. **17**: 20–26. 1939.

349. ROSE, F. T. Root-grafting rhododendrons. Gard. Chron. **99**: 71. 1936.

350. ROY, W. R. Studies of boron deficiency in grapefruit. Proc. Fla. State Hort. Soc. **56**: 38–43. 1943.

351. RUSCH, F. (Method of budding *Hevea* at the government coffee plantation "Bangelan"). Bergcultures **13**: 1233–1235. 1939. (Hort. Abst. **9**: 1415).

352. SAHUT, F. De l'affinité spécifique entre le sujet et le greffon. Rev. Hort. **57**: 149–152. 1885.

353. SALMON, E. S. and WARE, W. M. Interspecific grafting in *Humulus*. Gard. Chron. (London) **83**: 396. 1928.

354. SANSOME, F. W. Graft hybrids and induction of polyploids in *Solanum*. Rep. IX Int. Hort. Cong. 92–99. 1930.

355. Sass, J. E. Formation of callus knots on apple grafts as related to the histology of graft union. Bot. Gaz. **94**: 364–380. 1932.

356. Satina, Sophie. Periclinal chimeras in *Datura* in relation to the development and structure of the ovule. Am. Jour. Bot. **32**: 72–81. 1945.

357. ———— *et al.* Demonstration of the three germ layers in the shoot apex of *Datura* by means of induced polyploidy in periclinal chimeras. Am. Jour. Bot. **27**: 895–905. 1940.

358. ———— *et al.* Periclinal chimeras in *Datura* in relation to the development of the carpel. Am. Jour. Bot. **30**: 453–462. 1943.

359. Saunders, L. H. An experiment in the propagation of the kola. Trop. Agr. (Trinidad) **18**: 15. 1941. (Biol. Abst. **15**: 24, 265. 1941).

360. El Sawy, A. Characteristics of the main citrus stocks. Leafl. Hort. Sect. Egypt. **50**: 1–5, 1936. (Hort. Abst. **7**: 711. 1937).

361. Sax, K. The cause and permanence of size differences in apple trees. Me. Agr. Exp. Sta., Bull. 310. 1923.

362. Schmidt, A. Histologische Studien an phanerogamen Vegetationspunkten. Bot. Arch. **8**: 345–404. 1924.

363. Schmuck, A. A. *et al.* Alteration in the alkaloid composition due to the influence of stock upon scion in *Nicotiana*. Compt. Rend. (Doklady) Acad. Sci. U. R. S. S. **25**: 477–480. 1939.

364. Schrader, A. L. and Whitehouse, W. E. The budding and grafting of fruit trees. Md. Agr. Exp. Sta., Bull. 278. 1925.

365. Schuster, C. E. and Miller, P. W. A disorder of Persian (English) walnuts grafted on black-walnut stocks, resulting in girdling. Phytopath. **23**: 408–409. 1933.

366. Schwartzman, M. The carob tree and its cultivation in Palestine. Palestine Dept. Agr. & For., Bull. **1**: 1–48. (Biol. Abst. **10**: 11656. 1936).

367. Schweizer, J. (Mutual stock/scion effect in *Hevea*). Bergcultures **12**: 773–778. 1938.

368. Scott, L. E. A comparison of the performance of young Halehaven trees on Shalil and Carolina "natural" rootstocks. So. Car. Agr. Exp. Sta., Ann. Rep. **1942**: 131–138.

369. Sharrock, R. History of the propagation and improvement of vegetables. 1672.

370. Shaw, J. K. An investigation of the interrelation of stock and scion in apples. Proc. Am. Soc. Hort. Sci. **14**: 59–65. 1917.

371. ————. The Malling clonal stocks in relation to McIntosh and Wealthy. Proc. Am. Soc. Hort. Sci. **33**: 346–349. 1936.

372. ————. Budding ornamental *Malus* on the Malling rootstocks. Proc. Am. Soc. Hort. Sci. **38**: 661. 1941.

373. ———— and Southwick, L. A second report on some lethal rootstock-scion combinations. Proc. Am. Soc. Hort. Sci. **45**: 198–202. 1944.

374. Schmuck, A. *et al.* Formation of nicotine in plants grafted on tobacco. Compt. Rend. (Doklady) Acad. Sci. U. R. S. S. **32**: 365–368. 1941.

375. Siegler, E. A. and Bowman, J. J. Propagation of sour cherries by piece-root grafting to avoid spraying seedling stocks for leaf spot. Phytopath. **30**: 873–876. 1940.

376. Silberschmidt, K. Studien zum Nachweis von Antikörpern in Pflanzen. Planta. **13**: 114–168. 1931.

377. ————. Beiträge zur Kenntnis der Stoffwechselgemeinschaft zwischen Pfropfparten. Planta **19**: 729–780. 1933. (Biol. Abst. **9**: 4702. 1935).

378. ————. Weitere Versuche über die N-Verteilung in Pfropfungen und Stecklingen. Planta **22**: 1–22. 1934. (Biol. Abst. **11**: 2984. 1937).

379. ————. Die Verteilung proteolytischer Aktivität in den Stengelteilen von normalen Pflanzen und Pfropfungen. Planta **22**: 313–367. 1934. (Biol. Abst. 11: 3020. 1937).

380. ————. Die Abhängigkeit des Pfropferfolges von der systematischen Verwandtschaft der Partner. Zeit. Bot. **29**: 65–137. 1935.

381. Simon, S. V. Transplantationsversuche zwischen *Solanum melongena* und *Iresine lindeni*. Jahrb. Wiss. Bot. **72**: 137–160. 1930. (Biol. Abst. 5: 27163. 1931).

382. Sinclair, W. B. and Bartholomew, E. T. Effects of rootstock and environment on the composition of oranges and grapefruit. Hilgardia **16**: 125–175. 1944.

383. Singh, S. The choice of rootstocks for blood-red orange. Punjab Fruit Jour. **6**: 1174–1175. 1942. (Biol. Abst. **18**: 20301. 1944).

384. Sitton, B. G. and Dodge, F. N. Growth and fruiting of three varieties of pecans on different seedling rootstocks. Proc. Am. Soc. Hort. Sci. **36**: 121–125. 1938.

384*a*. Skoog, Folke, and Tsui, Cheng. Chemical control of growth and bud formation in tobacco stem segments and callus cultured in vitro. Am. Jour. Bot. **35**: 782–787. 1948.

385. Smirnov, V. and Smirnova, O. (Watermelons and melons for the north). Jarovizatsiia **3**: 66–70. 1939. (Biol. Abst. **14**: 15697. 1940).

386. Smith, C. O. The Japanese apricot as a root-stock. Proc. Am. Soc. Hort. Sci. **25**: 183–187. 1929.

387. Smith, W. W. Use of Malling and other dwarf apple rootstocks. N. H. Agr. Exp. Sta., Bull. 345. 1942.

388. Smyth, Elsie S. The seasonal cycles of ash, carbohydrate and nitrogenous constituents in the terminal shoots of apple trees and the effects of five vegetatively propagated rootstocks on them. II. Carbohydrate fractions and lignin. Jour. Pom. & Hort. Sci. **18**: 185–200. 1938.

389. Snyder, E. and Harmon, F. N. Vinifera grape scion influence on Dog Ridge stock. Proc. Am. Soc. Hort. Sci. **22**: 374–375. 1934.

390. ———— and ————. Hastening the production of fruit in grape hybridizing work. Proc. Am. Soc. Hort. Sci. **34**: 426–427. 1936.

391. Southwick, L. and Shaw, J. K. Further notes on the Malling clonal stocks in relation to McIntosh and Wealthy. Proc. Am. Soc. Hort. Sci. **36**: 133–137. 1939.

392. Spinks, G. T. Investigations on the variability of apple trees on seedling and on clone root-stocks. Long Ashton Res. Sta., Ann. Rep. **1936**: 50–65.

393. Strasburger, E. Ueber Verwachsungen und deren Folgen. Ber. Deut. Bot. Ges. **3**: 34–40. 1885.

394. ————. (Union of protoplasm). Jahrb. Wiss. Bot. **36**: 493. 1901.

395. ————. Zu der Atropinnachweis in den Kartoffelnollen. Ber. Deut. Bot. Ges. **24**: 599–600. 1906.

396. ————. Histologische Beitraege zur Vererbungsfrage. I. Typische und Allotypische Keimteilung. Jahrb. Wiss. Bot. **42**: 1–71. 1906.

397. Sudds, R. H. The comparative performance of several standard apple varieties on certain rootstocks, principally the clones Malling I, II, and XIII. Proc. Am. Soc. Hort. Sci. **36**: 311–318. 1939.

398. ———— and Marth, R. C. The effect of the rootstocks on nine years growth and yield of four apple varieties. Proc. Am. Soc. Hort. Sci. **42**: 326–334. 1943.

399. Summers, F. Improvement of yield in the para rubber tree. Rep. IX Int. Hort. Congr. 391–395. 1930.

400. Swarbrick, T. Some preliminary observations upon the influence of scion variety upon the root growth of young apple trees. Long Ashton Res. Sta., Ann. Rep. 42–49. 1927.

401. ————. The influence of the position of the top bud of the scion upon the stand of grafts. Long Ashton Res. Sta., Ann. Rep. 46–51. 1930.

402. ————. Rootstock and scion relationship. Some effects of scion variety upon the rootstock. Jour. Pom. & Hort. Sci. **8:** 210–228. 1930.

403. ———— and Naik, K. C. Factors governing fruit bud formation. IX. A study of the relation between leaf area and internode length in the shoots of Worcester Pearmain apple, as affected by six different vegetative rootstocks. Jour. Pom. & Hort. Sci. **10:** 42–53. 1932.

404. ———— and Roberts, R. H. The relation of scion variety to character of root growth in apple trees. Wis. Agr. Exp. Sta., Res. Bull. 78. 1927.

405. Swingle, C. F. Graft hybrids in plants. Jour. Hered. **18:** 73–94. 1927.

406. ————. Regeneration and vegetative propagation. Bot. Rev. **6:** 301–355. 1940.

407. Tanaka, T. and Tanaka, Y. (Propagation of citrus trees in Japan). (Studia Citrologica) **3:** 197–207. 1930. (Biol. Abst. **6:** 8000. 1932).

408. Thomas, H. E. *et al.* A transmissible leaf-casting yellows of peach. Phytopath. **30:** 322–328. 1940.

409. Thomas, L. A. Stock and scion investigations. III. The root-systems of some own-rooted apple trees. Jour. Coun. Sci. & Ind. Res. Australia **17:** 167–178. 1944.

410. Toenjes, W. Influence of some rootstocks on the Campbell Early grape. Mich. Agr. Exp. Sta., Quart. Bull. 26: 3–10. 1943.

411. Toxopeus, H. J. Stock-scion incompatibility in citrus and its cause. Jour. Pom. & Hort. Sci. **14:** 360–364. 1936.

412. Traub, H. P. and Auchter, E. C. Sprouting and grafting fractional parts of avocado embryos with attached cotyledonous material. Science **78:** 389–390. 1933.

413. Tufts, W. P. Correspondence, April 4, 1943.

414. Tukey, H. B. The importance of stocks in Kieffer pear growing. Jour. Hered. **19:** 113–114. 1928.

415. ———— and Brase, K. D. Influence of the scion and of an intermediate stem-piece upon the character and development of roots of young apple trees. N. Y. (Geneva) Agr. Exp. Sta., Tech. Bull. 218. 1933.

416. ———— and ————. Trials with pear stocks in New York. Proc. Am. Soc. Hort. Sci. **30:** 361–364. 1934.

417. ———— and ————. Size relationships of trees of five varieties of apple on several clonal rootstocks. Proc. Am. Soc. Hort. Sci. **37:** 299–304. 1940.

418. ———— and ————. Similarity in the nursery of several Malling Apple stock-and-scion combinations which differ in the orchard. Proc. Am. Soc. Hort. Sci. **39:** 245–246. 1941.

419. ———— and ————. The dwarfing effect of an intermediate stempiece of Malling IX apple. Proc. Am. Soc. Hort. Sci. **42:** 357–364. 1943.

420. ———— and ————. An incongeniality of the McIntosh apple when topworked onto Virginia crab. Proc. Am. Soc. Hort. Sci. **43:** 139–142. 1943.

421. ——— and ———. Differences of congeniality of two sources of McIntosh apple budwood propagated on rootstock U. S. D. A. 227. Proc. Am. Soc. Hort. Sci. **45**: 190–194. 1944.

422. ——— and ———. See differences in apple budwood. N. Y. (Geneva) Agr. Exp. Sta., Quart. Bull. 11: 9, 11. 1945.

423. Tydeman, H. M. New varieties of rootstock for plums. East Malling Res. Sta., Ann. Rep. **1934**: 100–102.

424. ———. The wild fruit trees of the Caucasus and Turkestan: Their potentialities as rootstocks for apples and pears. I. A first report on some wild quince from the Caucasus. East Malling Res. Sta., Ann. Rep. **1937**: 103–116.

425. ———. Further studies on new varieties of apple rootstocks. Jour. Pom. & Hort. Sci. **20**: 116–126. 1943.

426. Uphof, J. C. T. Wissenschaftliche Beobachtungen und Versuche an Agrumen. VIII. Morphologische Studien an einem Pfropfbastard zwischen der Apfelsine Pineapple (*Citrus sinensis* Osbeck) und der Bigarde (*C. aurantium* L.) Gartenbauwiss. **9**: 421–426. 1935. (Hort. Abst. **6**: 133).

427. Upshall, W. H. Dwarf pear trees. Canad. Hort. & Home Mag. **63**: 36, 41–42. 1940.

428. ———. Compatability of Bechtel's Crab on some Malling root stocks. Sci. Agr. **21**: 687–688. 1941.

429. ———. Malling stocks and French crab seedlings as stocks for five varieties of apples. III. Sci. Agr. **23**: 537–545. 1943.

430. Vaidya, V. G. The seasonal cycles of ash, carbohydrate and nitrogenous constituents in the terminal shoots of apple trees and the effects of five vegetatively propagated rootstocks on them. I. Total ash and ash constituents. Jour. Pom. **16**: 101–126. 1938.

431. Vaile, J. E. The influence of rootstocks on the yield and vigor of American grapes. Proc. Am. Soc. Hort. Sci. **35**: 471–474. 1937.

432. Van Kleef, A. Grafting rhododendrons. Florist's Exchange **51**: 383. 1924.

433. Van Overbeck, J. and Gregory, L. E. A physiological separation of two factors necessary for the formation of roots on cuttings. Am. Jour. Bot. **32**: 336–341. 1945.

434. Varma, S. R. A novel mango graft. Punjab Fruit Jour. **5**: 957. 1941. (Biol. Abst. **17**: 2761. 1943).

435. Vöchting, H. Ueber Transplantation am Pflanzenkörper. 1892.

436. ———. Ueber die durch Pfropfen herbeigeführte Symbiose des *Helianthus tuberosus* und *Helianthus annuus*. Sitzber. Preus. Akad. Wiss. Berlin: 705–721. 1894.

437. Volk, A. Beiträge zur Kenntnis der Wechselbeziehungen zwischen Kulturpflanzen, ihren Parasiten und der Umwelt. 4. Herausg. von E. Schaffnit. Einflüsse des Bodens, der Luft und des Lichtes auf die Empfänglichkeit der Pflanzen für Krankheiten. Phytopath. Zeits. **3**: 1–88. 1931. (Biol. Abst. **7**: 15354. 1933).

438. Von Wettstein, F. and Pirschle, K. Über die Wirkung heteroplastischer Pfropfungen und die Übertragung eines Gen-bedingten Stoffes durch Pfropfung bei *Petunia*. Biol. Zentr. **58**: 123–142. 1938.

439. Vyvyan, M. C. The effect of scion on root. III. Comparison of stem and root-worked trees. Jour. Pom. **8**: 259–282. 1930.

440. ———. The distribution of growth between roots, stems, and leaves in a young apple tree and its possible bearing on the problem of stock effect on tree vigour. East Malling Res. Sta., Ann. Rep. **1933**: 121–131.

441. ———. Relative influence of rootstock and of an immediate piece of stock stem in some double grafted apple trees. Jour. Pom. **16**: 251–273. 1938.

442. ————. Notes on the effect of the variety of stock used to provide the intermediate stem-piece in some double-working of apple trees. East Malling Res. Sta., Ann. Rep. **1938**: 148–156.

443. Wagner, S. Ein Beitrag zur Züchtung des Topinambur und zur Kastration bei *Helianthus.* Zeits. Zucht. Reibe A, Pflanzenzücht. **17**: 563–582. 1932. (Biol. Abst. **8**: 6670. 1934).

444. Warne, L. G. G. and Raby, Jane. Water conductivity of the graft union in apple trees with special reference to Malling rootstock No. IX. Jour. Pom. **16**: 389–399. 1939.

445. ———— and Wallace, T. The composition of terminal shoots and fruits of two varieties of apple in relation to rootstock effects. Jour. Pom. **13**: 1–31. 1935.

446. Waugh, F. A. The graft union. Mass. Agr. Exp. Sta., Tech. Bull. 2. 1904.

447. Webber, H. J. and Barrett, J. T. Root-stock influence in citrus. Rep. IX Int. Hort. Cong. 358–373. 1930.

448. Webster, C. C. Topworking *Alcurites Montana* Nyasaland Agr. Quart. Jour. **2**: 10–15. 1942. (Biol. Abst. **17**: 14516. 1943).

449. Weimer, J. L. Alfalfa dwarf, a virus disease transmissible by grafting. Jour. Agr. Res. **53**: 333–347. 1936.

450. Weinard, F. F. and Dornfr, H. B. *Rosa odorata* as a grafting stock for indoor roses. Ill. Agr. Exp. Sta., Bull. 290. 1927.

451. Weiss, F. E. The problem of graft hybrids and chimaeras. Biol. Rev. **5**: 231–271. 1930.

452. Went, F. W. Transplantation experiments with peas. III. Bot. Gaz. **104**: 460–474. 1943.

453. Whitaker, T. W. *et al.* Studies on the precipitin reaction in plants. IV. The question of acquired reactions due to grafting. Am. Jour. Bot. **20**: 297–308. 1933.

454. White, P. R. Developmental responses in isolated plant tissue systems. Growth **6**: 55–71. 1942.

455. ————. Metastatic (graft) tumors of bacteria-free crown-galls in *Vinca rosea.* Am. Jour. Bot. **32**: 237–241. 1945.

456. Winkler, H. Ueber Pfropfbastarde und Pflanzliche Chimaeren. Ber. Deut. Bot. Ges. **25**: 568–576. 1907.

457. ————. *Solanum tubingeuse,* ein echter Pfropfbastard zwischen Tomate und Nachtschatten. Ber. Deut. Bot. Ges. **26a**: 596–608. 1908. (Graft hybrids. Bot. Gaz **47**: 250. 1909. R. R. Gates).

458. ————. Ueber die Nachkommenschaft der Solanum Pfropfbastarde und die Chromosomenzahlen ihrer Keimzellen. Zeits. Bot. **2**: 1–38. 1909.

459. ————. Untersuchen ueber Pfropfbastarde. I. Die Unmittelbare Gegenseitige Beeinflussung der Pfropfsymbionten. 1912.

460. ————. Die Chimaerenforschung als Methode der Experimentellen Biologe. Sitz. Phys.-Med. Ges. Wurzburg 95–119. 1913.

461. ————. Transplantation Pfropfung. Pfropfbastarde. Handw. der Naturwissenschaften **10**: 18–29. 1915.

462. ————. Ueber die Experimentelle Erzeugung von Pflanzen mit Abweichenden Chromosomenzahlen. Zeits. Bot. **8**: 417–531. 1916.

463. ————. Über zwei Solanum—Chimären mit Burdonenepidermis. Planta **21**: 613–656. 1934.

464. ————. Chimaeren und Burdonen. Die Biologie **9**: 279–290. 1935.

465. Withrow, Alice P. and Withrow, R. B. Translocation of the floral stimulus in *Xanthium.* Bot. Gaz. **104**: 409–416. 1943.

466. Wright, J. S. Cell union in herbaceous grafting. Bot. Gaz. **18**: 285–293. 1893.

467. Yampolsky, C. Male-female grafts in *Mercurialis annua.* Jour. Hered. **21**: 65–72. 1930.

468. Yasudo, S. An experiment to graft the style upon the ovary in *Petunia violacea*. Proc. Imp. Acad. (Tokyo) **7**: 72–75. 1931. (Biol. Abst. **6**: 24551. 1932).

469. Yeager, A. F. Xylem formation from ring grafts. Proc. Am. Soc. Hort. Sci. **44**: 221–222. 1944.

470. Yerkes, G. E. Bridge grafting. U. S. Dept. Agr., Farm. Bull. 1369. 1923.

471. ————. Propagation of trees and shrubs. U. S. Dept. Agr., Farm. Bull. 1567. 1929.

472. ————. Rose under-stock in a five-year test. Proc. Am. Soc. Hort. Sci. **27**: 463–466. 1930.

473. ———— and Anthony, R. D. Seedling apple stocks of known origin in nursery and orchard tests. Proc. Am. Soc. Hort. Sci. **38**: 331–335. 1941.

474. ———— and Sudds, R. H. The effect of the stocks on seven years' growth of four apple varieties. Proc. Am. Soc. Hort. Sci. **37**: 294–297. 1939.

475. Zakharova, E. I. and Potapenko, Ya. I. Development of seedling scions grafted to the crown of bearing apple trees. [Russian]. Proc. Lenin Acad. Agr. Sci. Moscow **14**: 3–6. 1939.

476. Zebrak, A. R. (Grafting in genetically different pea lines). Trudy Timirjaze. Seljskohoz. Acad. I: 88–97. 1935. (Herb. Abst. **7**: 240. 1937).

477. ————. (Grafting experiments with herbaceous plants). Bull. Vsesojuz. Akad. S-H Nauk. No. *9*: 17–22. 1936. (Herb. Abst. **7**: 240. 1937).

478. ————. (Intergeneric and interfamily grafting of herbaceous plants). Timirjazev Seljskohoz. Akad. *2*: 115–133. 1937. (Herb. Abst. **9**: 674. 1939).

479. Zimmerman, P. W. and Connard, Mary H. Reversal of direction of translocation of solutes in stems. Contr. Boyce Thompson Inst. **6**: 297–302. 1934.

THE REACTIONS OF BACTERIAL VIRUSES WITH THEIR HOST CELLS[1]

THOMAS F. ANDERSON
University of Pennsylvania

THE NATURE OF THE VIRUS PROBLEM

In its early stages the study of a sector of nature may be divided into two branches. In one we inquire what an object under investigation *is;* we want to know its morphology and its relation to other more or less similar objects. In the other branch we study what the object *does* and how it came to be what it is. Thus in chemistry we have molecular structure on the one hand and kinetics of reaction on the other. In biology, anatomy and embryology may be taken as examples of the two points of view. As a science progresses certain relations between structure and function begin to emerge, and eventually the pursuit of these relations may become the major interest in the field.

The study of viruses is a very young science indeed. In recent years much progress has been made toward a better understanding of those phases of the virus problem concerned with the infectious agent or virus particle (7). Improved methods of isolation, culture, concentration and assay have made purified preparations of many viruses available in relatively large quantities for physical and chemical characterization. Application of the ultracentrifuge and diffusion techniques gives indirect evidence of the mean sizes and shapes of virus particles in solution; the electron microscope has made possible direct observation of sizes and shapes of individual particles in the dry state (7, 8, 53, 56, 68, 69, 72); and x-ray diffraction has given clues to the atomic architecture of certain plant virus particles (11). It has turned out that this segment of biological material includes particles as small as 10 mμ and as large as 200 mμ in diameter. The morphologies of the viruses range from relatively large cell-like structures (vaccinia), some of them being tadpole-shaped (T2 bacteriophage), to simple rods (tobacco mosaic) and spheres (bushy stunt). On the other hand, the particles of any particular strain of virus exhibit a remarkable uniformity in size and structure.

---

[1] This work was supported by Contract N6 ori 168 TO II between the Office of Naval Research and the Trustees of the University of Pennsylvania.

By themselves the virus particles seem to be quite inert; like the seeds and spores of higher forms, whose regularity in size and shape they resemble, they become active only in a suitable environment—the host cell. Attempts to study their mode of reproduction by grinding up host cells at intervals after infection have yielded only debris, and in some cases a few finished virus particles. Direct observation of their mode of reproduction is impossible, since, on the one hand, the particles themselves are smaller than the wave lengths of visible light, and, on the other, most cells are too thick for convenient observation of internal structure with the electron microscope. Investigators of the mode of virus reproduction are therefore forced to employ the more indirect methods of genetics, biophysics and biochemistry.

With the biochemical approach we should like to observe the effects of various nutrients and specific enzyme poisons on virus-producing systems. The geneticist would study the mechanism of inheritance—he will be concerned with the aberrations in virus production (mutations) which yield new types; he will attempt to break down the virus into semi-independent units of inheritance as he has the genes of higher forms; he will study the conditions under which units can combine or be exchanged to yield new hybrid types. Basic to these problems lies that of the deployment of host and virus elements on a molecular and indeed atomic level which leads to infection and virus production. Here are needed the tools and methods of analysis of biophysics. The intricacies of the problem cannot be overestimated; the program is an ambitious one.

An analysis of the multiplication of plant or animal viruses is difficult because of the complex nutrient media (sap, body fluids) in which the cells, which are the real virus hosts, are bathed. Moreover, the great variety of cells which compose the tissues makes it difficult to distinguish the direct effect which the highly selective virus produces on the particular type of cell on which it acts, from the indirect effects on neighboring cells and on the organism as a whole. Only in the bacteriophages are such complications largely absent. Here one may work with single undifferentiated clones at dilutions which, as far as we know, eliminate the possibility of interactions between cells. The nutrition supplied in the medium can be varied at will. Moreover, in this system the method of virus assay are as simple, rapid and precise as the methods of counting colony-forming cells of the bacterial host. Then, too, the short generation time of the host makes it possible to complete in 24 hours experiments on growth and heredity which involve huge clones of individual cells (10^{11} or more) or of virus particles. For these and other reasons it is mainly in the bacterial viruses that we have the type of information on virus reproduction which will be discussed in this review.

Most of this work has been reported in the last two or three years. A number of excellent reviews of earlier work on bacterial viruses is available (13, 23, 28). The ecological aspects of animal viruses (14), their physical and chemical properties (7, 74) and details of the course of infection by poliomyelitis (42) have been reviewed recently. The chemistry of the plant viruses (45) and what is known of their action (10) has also been reviewed in recent years and will not be referred to here. At present it is difficult to make a clear cut distinction in kind between genes, plasmagenes, cytogenes, transforming principles and viruses (21); recent developments in these fields (59) will therefore be watched with interest by the student of viruses (*cf.* papers in Cold Spring Harbor Symp. Quant. Biol., Volumes 11 and 12, 1946 and 1947).

GENERAL PROPERTIES OF THE BACTERIAL VIRUSES

Discovery. In 1915 F. W. Twort (71) reported the observation of bacterial colonies which appeared to dissolve slowly, and of holes which arose on bacterial smears on solid media. He found that material taken from these clear areas could induce the same spreading lytic action when applied to normal smears and that the lytic principle could multiply indefinitely on these bacteria. In 1917 F. d'Herelle (38, 39) made a similar set of observations and eventually showed that the bacteriophages, as he called the lytic principles, were particulate in nature, for the number of "plaques", as he termed the holes they produced on a bacterial smear, is directly proportional to the amount of the lytic principle applied uniformly to the smear. Both investigators showed that the lytic principle was extremely small, since it passed through filters which would hold back all visible bacteria.

Collection and Assay. To acquire a set of phages active against a certain bacterium, one first collects material from regions in which the prospective host is present and being destroyed in great numbers—feces, sewage or, if possible, exudates from infections caused by the organism (39). These samples are filtered to remove bacteria, then mixed with a dilute suspension of host cells and incubated for 24 hours to allow the bacteriophages which are active on the prospective host to multiply. The culture is then filtered again and plated at various dilutions with about 10^8 bacteria on solid agar. If one is lucky in his search, he will find that after incubation of the plates a number of clear areas have appeared on the otherwise uniform smear of the growing host bacteria. Each area presumably contains a clone of virus particles, so one takes each of them up separately in liquid medium and filters each again to remove the bacteria. Now one adds a sample from each of the filtrates #1, 2, . . . *etc.* to each of a corresponding number of dilute bacterial suspensions in nutrient broth. After two to eight hours of incubation with aeration, the bacteria in most of the tubes will have disappeared. The resulting clear suspensions should then be filtered to yield virus suspensions called "lysates". Such lysates will usually contain between 10^7 and 10^{11} plaque-forming particles per cc. They may be assayed by plating appropriate dilutions with an excess of the host bacteria; then the number of plaques which appear after 18 hours incubation, divided by the volume of the initial lysate which was applied to the plate, is proportional to the concentration of virus particles in the lysate.

Resistant Strains of the Host. Characterization of Bacteriophages. If one plates 10^6 or more virus particles with about 10^8 bacteria, the virus will lyse almost all the bacteria and permit a few colonies of resistant bacteria to grow. We thus have available a set of "indicator" strains of bacteria, denoted B/1, B/2, *etc.*, each of which is resistant to the virus in the corresponding lysate. The indicator strains have been shown to arise by mutation of the host in the absence of the virus (55, 32).

The next step is to determine whether the several lysates contain the same or different viruses. Different viruses frequently form plaques which differ in size, presence or absence of a halo, *etc.* Also, in general, each of the viruses will be active, *i.e.*, form plaques, on a *different* set of the resistant bacteria. If virus #1 behaves exactly as the virus in lysate #15, say, in its activity against the spectrum of resistant bacteria, and the two form indistinguishable plaques, especially when they overlap on the same plate (41), they are probably the same virus.

Host-Range Mutations. If one plates a large excess (10^9 particles) of virus #1, say, with bacteria which are resistant to the main bulk of virus in the lysate #1, he will usually observe a few plaques on the smear of B/1. These plaques are considered (48) to have arisen from virus particles which in their formation have mutated to activity on the cells which are resistant to the parent virus. Thus we obtain a collection of hosts, bacterial viruses and their mutants suitable for further study.

With perseverence one could collect a hundred or more demonstrably different bacteriophages active against a single strain of bacterium. Since this is too many for the intensive type of study we wish to discuss in this review, a group of workers in this country has limited its attention to a set of seven "wild type" viruses, designated T_1, T_2, . . . T_7, all active on a single non-motile strain, "B", of *E. coli.* Demerec and Fano (32) collected these viruses and renamed some of them. In the earlier literature, $T_1 = \alpha =$ P28; $T_2 = \gamma =$ PC. Although gathered from different sources, T_7 and the virus δ seemed to be identical and the latter was discarded.

Life Cycle and Morphology. The life cycle of these bacterial viruses consists of three easily recognizable stages (23):

a) Adsorption of the virus on the host cell.
b) A process of proliferation on the virus within the host.
c) Lysis of the host with simultaneous liberation of new virus.

An examination of these three stages with the electron microscope serves the two-fold purpose of telling us something of their nature and of enabling us to identify the virus particles. In Fig. 1 and Fig. 2 we see viruses T_2 and T_4 adsorbed on bacteria which appear as watermelons in comparison to the tadpole-shaped virus particles (53). Following adsorption, no change in the morphology of the host is to be seen, nor does the number of visible virus particles increase. The intracellular events leading to virus liberation are not visible. However, beginning 21 minutes after adsorption, bacteria may be seen which have burst, as in Fig. 3, to liberate a hundred or so phage particles. These are quite like the infecting one in structure.

In this way the infectious particle of each virus in the T set has been identified. Two, T_4 and T_6, which form small plaques, are

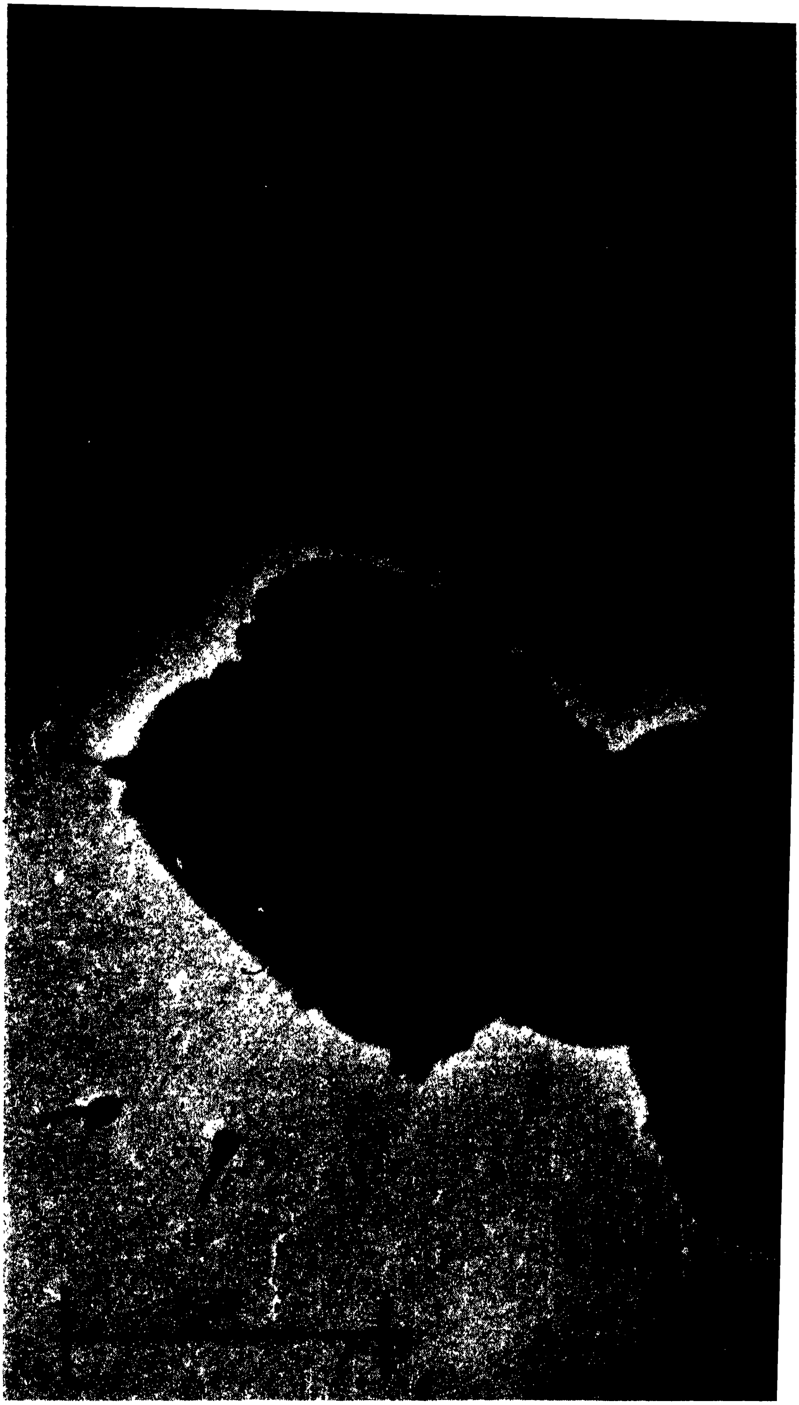


FIG. 1. *E. coli,* strain B, with adsorbed tadpole-shaped particles of virus T_2. The specimen was prepared five minutes after mixing the virus and host cells. EMG. 528 a × 40,000, (53).

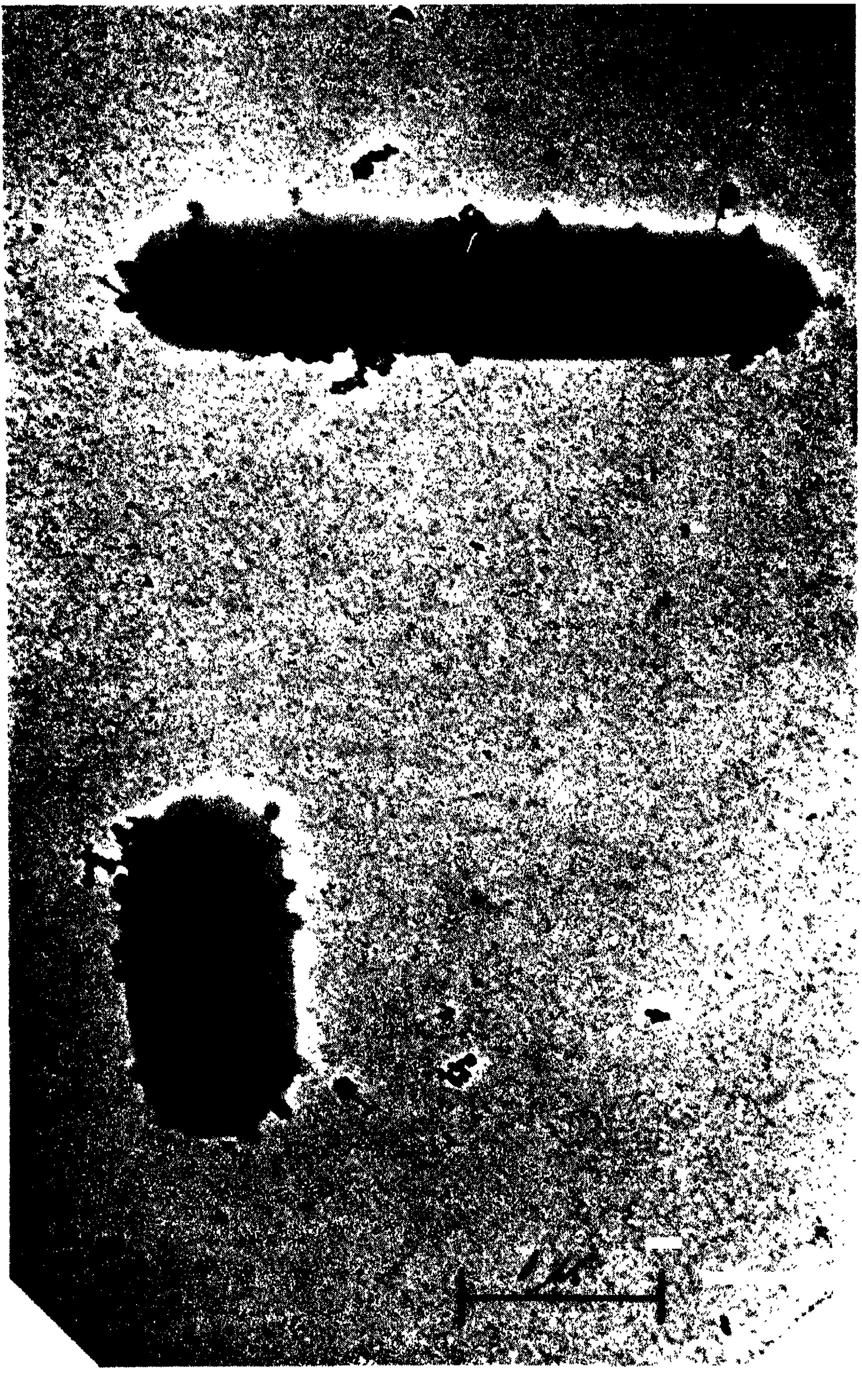


FIG. 2. *E. coli*, strain B, with adsorbed tadpole-shaped particles of virus T_4. The specimen was prepared five minutes after mixing the virus and its host cells in a solution of *l*-tryptophane Without the tryptophane to activate the virus, little adsorption occurs. As described in the text, various stages in the conjugation of virus with host cells may be discerned. EMG. 169 d × 24,000. (Anderson, unpublished).

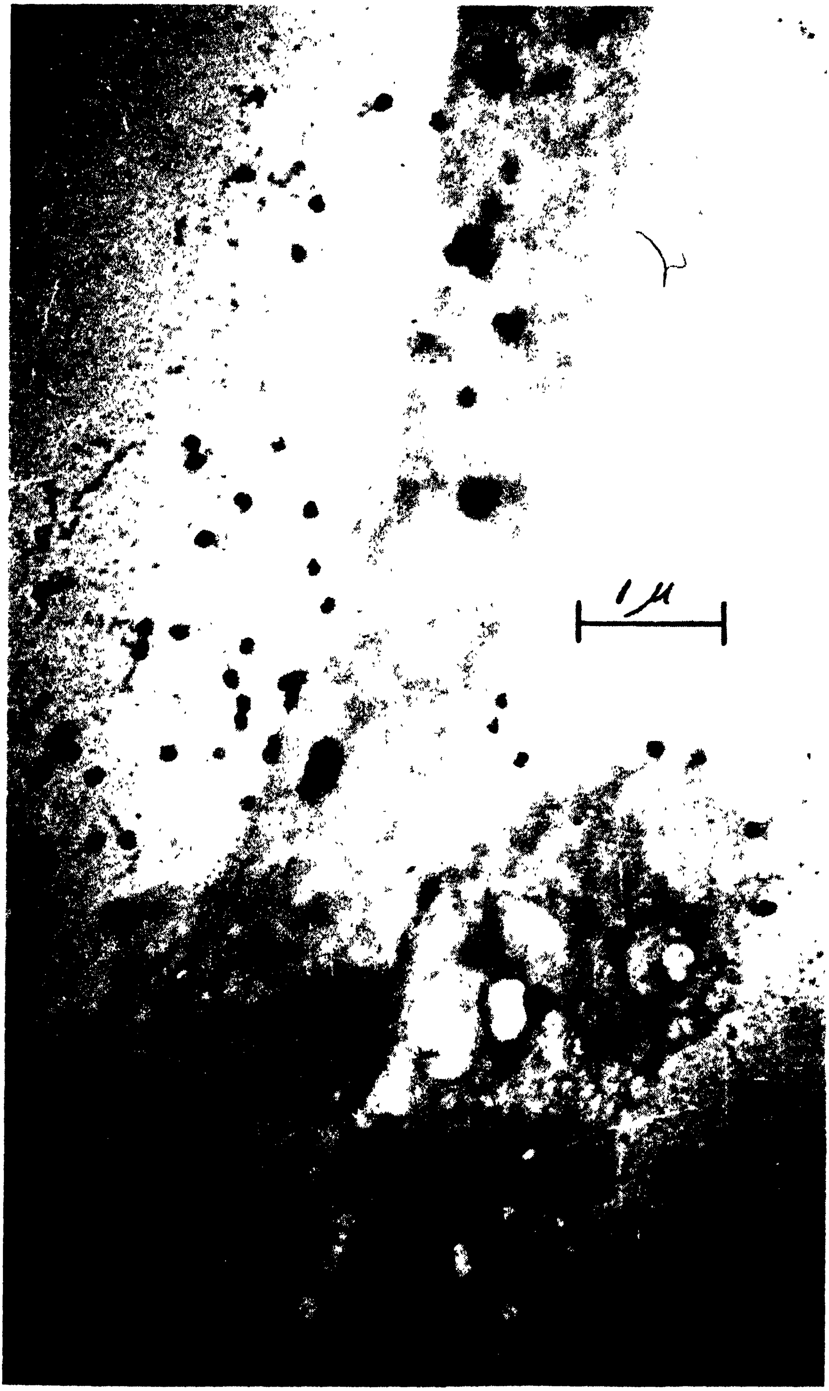


FIG. 3. Lysing virus-host complexes of *E. coli*, strain B, and the virus T_2. The specimen was prepared 21 minutes after mixing the virus and host cells. Many newly liberated tadpole-shaped T_2 particles are seen together with lytic debris. EMG. 790 b × 18,000, (56).

like T_2, with heads about 800 Å in diameter, containing a dense internal structure, and to which a well-defined "tail" about 1000 Å long is attached (7). T_3 and T_7, which form large plaques, are both small spheres about 450 Å in diameter (28). T_1, which also forms large plaques, is such a sphere, but with a faint tail about 1000 Å long (53, 56). T_5 forms small plaques and has a head about Å in diameter with a long faint tail (7). Thus the large viruses form small plaques, and the small viruses form large plaques, as had been inferred from earlier more indirect measurements of particle sizes.

Two additional physical properties of the bacteriophages seem to be correlated with their morphologies. Thus the large viruses T_2, T_4, T_6 and T_5 are much more sensitive to sonic vibration than the small viruses T_1, T_3 and T_7 (*7d*). Furthermore, the similar viruses, T_2, T_4 and T_6, which appear in the electron microscope to have membranes surrounding the internal structures of the heads, can be disintegrated by what may be termed "osmotic shock" (*7e*). If these viruses are incubated with 4M NaCl and the suspension then diluted *rapidly* with water, the viruses are inactivated, while little inactivation occurs if the suspensions in salt solution are gradually brought to lower ionic strengths. Electron micrographs of the latter preparations revealed normal particles, while micrographs of the former, rapidly diluted preparations revealed virus "ghosts"—empty head membranes with tails attached. These results are compatible with the view that the membranes of the even-numbered viruses, like those of cells, are somewhat permeable to NaCl but much more permeable to water molecules. Like cells the virus heads swell when the ambient salt concentration is reduced, and actually burst if the reduction is sufficiently large and sudden. Osmotic shocks which inactivate the even-numbered viruses have no detectable effect on the small viruses T_1, T_3 and T_7 which appear in electron micrographs not to have membranes.

In spite of the morphological similarity of some of the viruses in the T set, each has a distinctive pattern of activity against the various resistant strains of the host (32).

Serological Properties of Bacteriophages. If one injects a virus preparation into a rabbit, the animal's serum acquires the ability to neutralize the virus in vitro, *i.e.*, render it non-infectious. Different viruses possess their antigenic power in varying degrees.

Unlike the influenza viruses which react with antisera to the hosts from which they came (44), the bacterial viruses are serologically distinct from their hosts. As far as is known, antiserum to a bacterium does not react with the phages which are active on that bacterium.

Although the mechanism of the neutralization reaction is unknown, it may be supposed that antibody molecules in the antiserum, when adsorbed on the virus antigens, cover up and render inactive certain prosthetic groups on the virus surface. Neutralized phage can be reactivated by removal of the antibody by a proteolytic enzyme such as papain (43). Antibody molecules may have more than one such point of attachment (valence) and so form bridges between virus particles and cause them to aggregate. Antibody to phage does not agglutinate host bacteria. On the other hand, if each bacterium in a suspension has adsorbed a number of phage particles, the bacteria can be agglutinated by phage antibodies which presumably form bridges between the adsorbed virus particles. Burnet *et al* (15) have demonstrated that phage which has been just neutralized by a small amount of antiserum is still capable of "coating" susceptible bacteria in this way, rendering them agglutinable by an excess of antibodies to the phage. From this it would appear that the primary action of antiserum in neutralizing phage is not on those virus elements which are responsible for specific adsorption on the host.

It was clear ten years ago (15) that serological tests provide the most straightforward classification of bacteriophages. Now that we know the morphologies of the phages, this is even more apparent. Viruses in the T set which have similar morphologies cross-react serologically. Thus antiserum against T_2 inactivates only T_2, T_4 and T_6. Antiserum against T_3 inactivates only T_3 and T_7. An antiserum against T_1 or one against T_5 fails to affect any of the other viruses in the T set; T_1 and T_5 are the sole representatives of their orders in the set (24, 28).

Many viruses which are active on other types of bacteria have morphologies strikingly similar to those of the T set. Thus a staphylococcus phage resembles T_5 in structure (56). It would be interesting if viruses which are morphologically similar but active on greatly different organisms should be found to have serological properties in common. In higher organisms serological relationships would be taken to imply phylogenetic relatedness between forms.

Multiplicity of Infection. In many types of experiment it is important to have reliable information on the distribution of virus particles among the infected bacteria. If a number of virus particles, s, is adsorbed on a number of bacteria, t, the ratio s/t is termed the "multiplicity of infection," m. If we assume that all the bacteria have the same *tendency* to adsorb virus irrespective of the number already adsorbed, and that in the experimental procedure they all are given *equal chances* to adsorb virus, then the distribution of the virus particles among the bacteria can be shown to follow the classical law of Poisson (62). This law predicts that the fraction of bacteria, P_r, which have adsorbed exactly r virus particles, will be

$$P_r = \frac{m^r e^{-m}}{r!} \qquad (1)$$

The multiplicity may be determined either by determining s and t directly, *i.e.* by counting virus and bacteria, or by determining the fraction of bacteria, P_0, which have adsorbed *no* virus and which can therefore form colonies (54). Thus

$$P_0 = e^{m} \text{ or } m = \log_e P_0.$$

The first method is most accurate when m is less than 1; both may be used when m is 1 or greater. The fact that the two methods agree where $1 < m < 10$ is justification for using such calculations, and suggests that in this region the assumptions are essentially correct.

When $m << 1$ a very small percentage of the bacteria will have adsorbed more than one virus particle. In such cases studies of virus growth are concerned only with "singly infected bacteria." We speak of "multiple infection" when a considerable proportion of the bacteria have adsorbed more than one virus particle. For example, when $m = 3$, 80% of the bacteria will have adsorbed two or more virus particles:

$$P_0 = \frac{3^0 e^{-3}}{0\,!} = 0.05; \qquad P_1 = \frac{3^1 e^{-3}}{1\,!} = 0.15;$$

$$P_2 + P_3 + \dots P_\infty = 1 - P_0 - P_1 = 0.80$$

In such a case virus growth occurs primarily in multiply infected cells. As we shall see, the use of such probability calculations is becoming increasingly important in the interpretation of much of the experimental work on bacterial viruses (52).

One Step Growth Curves. Average and Individual Burst Sizes. The average number of virus particles liberated per infected bacterium in a culture is termed the "average burst size." It is determined by an analysis of "one step growth curves" obtained as follows (31, 34). At time zero, a predetermined number of virus particles is added to an aerated culture of host cells, and a period (5 min., say) is allowed for the virus to be adsorbed on the cells. Antivirus serum is then added. It inactivates the free virus but not the adsorbed virus (26). A sample is then diluted 1:1000 or more, and assayed at intervals for the total number of infectious centers of plaque-forming bodies. At a time called the "latent period", which is characteristic of the virus, growth conditions, *etc.*, the number of infectious centers will be found to rise sharply and eventually level off after all the infected bacteria have burst and liberated virus. The average burst size is simply taken to be the ratio of the final virus titer to the number of bacteria which had initially been infected. Since the average burst size varies with the stage of growth and nutrition of the host, it is extensively used as an index to events which occur within the infected cell. It must be admitted, however, that the evidence thus obtained is somewhat indirect. For example, one-step growth curves for single infection and multiple infection displayed the same latent periods and burst sizes (24, 31). This led all workers in the field into believing that only one virus particle was effective in multiple infection. As we shall see, this conclusion proved to be erroneous.

The number of virus particles liberated from individual bacteria can also be determined. Here the adsorption mixture is highly diluted and divided *before lysis occurs* into such small samples that only 50% of them contain an infected bacterium. After they have lysed, the total number of virus particles in each sample is determined by counting the plaques it produces when it is plated. The individual burst sizes from B infected with T_1 ranged from less than 25 to more than 1000, the average agreeing, as it should, with that obtained from one-step growth experiments (25). Similar variations are observed in individual bursts of other viruses and cannot be correlated with the sizes of the infected host cells.

The possibility that the wide variation in burst size might be due to heritable variations in the bacterial population or in the infecting virus particles themselves has not been examined.

We shall add many more examples of clues to the mechanism of virus production which, because we have no very clear picture of the processes involved, seem to be arbitrary and disconnected. Yet in the final analysis, all of them, even those which now seem trivial, will have to fit together. The most easily accessible set of these clues comes from studies of the first step in the virus attack on the host cell—adsorption.

FORMATION OF THE VIRUS-HOST COMPLEX: ADSORPTION

It is clear that a mixture of virus and susceptible host cells will in general contain two kinds of plaque-forming particles or infectious centers: the infected bacteria and the free unadsorbed virus particles. Since each will form only one plaque when plated on suitable media, some means of distinguishing between them must be employed if one wishes to determine the extent of virus adsorption. The most obvious method, perhaps, depends on the difference in size—centrifuging the mixture at 4000 rpm for 5 to 15 minutes, depending on the volume to be treated, will throw down the infected bacteria and leave the free virus in the supernatant liquid (22). Another method makes use of the fact already mentioned that the intracellular virus is not affected by an antiserum to the virus (26). Addition of antivirus serum to a mixture of virus and host cell, therefore, inactivates the free virus, leaving only the infected cells to form plaques when the suspension is diluted and assayed. Other methods of measuring the extent of adsorption might well be developed which depend on a difference in susceptibility to chemical or physical treatments. For example, under some conditions the T_4-host complex is inactivated by 5-methyl tryptophane, while the free virus is not (20). Usually the virus-host complex is more susceptible to killing by heat than is the free virus. On the other hand, the complex may be much more resistant to ultraviolet irradiation than the free virus, as will be discussed in greater detail in a later section.

Elecron micrographs of mixtures of virus and host seem to show a number of stages in the adsorption process. Specimens prepared five minutes after mixing T_2 or T_4 virus and their host cells contain some particles which appear to be just touching a host cell, but are otherwise visibly unchanged (Figs. 1 and 2). Others have their tails missing, the heads resting against the host. The recognizable parts of still others are mere blebs on the host cell wall; most of the internal structure in the heads of these particles has disappeared, presumably into the protoplasm of the host. At some point in the adsorption process, the reaction becomes essentially irreversible. This is the point at which the virus is said to be "adsorbed" in a physiological sense.

Rates of Adsorption. If one measures the amount of virus adsorbed at various times after mixing virus and host cells, it is usually found (22, 65, 66) that the free phage concentration (P) decreases exponentially in the early stages of adsorption. Increasing the bacterial concentration (B) increases the rate proportionally, as though the reaction were a simple combination of phage with the host cells:

$$B + P \xrightarrow{K_1} B{:}P \qquad (2)$$

The rate of decrease of free phage in the mixture follows the differential equation for a bimolecular reaction:

$$\frac{-d(P)}{dt} = K_1 \ (B)(P) \qquad (3)$$

and the concentration of free phage decreases with time according to the equation:

$$-2.3 \log \frac{(P)}{(P)_0} = K_1 \ (B)t \qquad (4)$$

when $(P)_0$, the concentration of free phage at the start of the reaction, is much less than the concentration of host bacteria.

As adsorption continues to the point where about 99% of the virus has been adsorbed, it is usually found that the rate of adsorption falls off rather abruptly (22, 65). When this residual slowly adsorbed fraction is separated from the infected bacteria in the adsorption mixture, its rate of adsorption on fresh bacteria is found to remain low, at least for the hour or so required for the experiments. This residual fraction seems to contain virus of the same genetic type as the main fraction, since its offspring have the same rate of adsorption as the bulk of the original virus. The nature of the inhibition of adsorption remains a mystery, but it should be noted that electron micrographs of filtered lysates show many virus particles attached to what appear to be small shreds of bacterial membranes. Such fragments would have a much slower rate of sedimentation than the bacteria and would presumably be counted with free phage in experiments cited if the particles eventually were able to free themselves to infect host cells and form plaques on a bacterial smear. A study of the factors which influence the rates of adsorption of these residual fractions might contribute to our knowledge of the mechanism of virus penetration or of lysis or both.

Assuming the diffusion (Brownian motion) alone determines the number of collisions between phage and host cell, and that each hit leads to adsorption, one obtains (66) from von Schmoluchowsky's theory of coagulation (67) a relation between the adsorption rate constant, K_1, the effective radius of the bacterium, r, and the rate of diffusion of the phage, D:

$$K_1 = 4\pi Dr \tag{5}$$

The observed rate constants K_1 for the initial stages of adsorption depend on a number of factors, including the type of phage, the salt concentration and the physiological state of the host bacteria, being as high as 300×10^{-10} cc./min. for actively growing motile bacteria, and as low as 5×10^{-10} for bacteria which are nearing the stationary stage of maximum growth (22). Schlesinger (66), using a phage similar to T_2 and active on a non-motile strain of E. coli, obtained $K_1 = 20 \times 10^{-10}$ cm.^{3}/min., from which he estimated the rate of diffusion D to be 6.3×10^{-8} cm.^{2}/sec. Then, assuming the particles to be spherical, he calculated the effective diameter of the phage, d, by the Einstein relation:

$$d = \frac{RT}{3\pi N\eta D} \tag{6}$$

where R is the gas constant, T is the absolute temperature, N is Avagadro's number, and η is the viscosity. This gave the value for the diameter:

$$d = \frac{8.32 \times 10^7 (273 + 37)}{3\pi \,.\, 6.06 \times 10^{23} \times 0.007 \times 6.3 \times 10^{-8}} = 10.2 \times 10^{-6} \text{ cm.}$$

in good agreement with the average diameter of such a particle as observed years later with the electron microscope. On the basis of accepted theories of virus adsorption it is most surprising that this and similar calculations which can be made with more recent data should yield a value for the particle diameter which is anywhere near the correct order of magnitude. To understand this it might be useful to describe the current theory of adsorption and discuss its implications.

Receptor Spot Theory. The highly specific adsorption of a virus on its host cell has been generally ascribed to the steric fitting of a specific and therefore complicated surface configuration on the virus particle to a complementary structure (receptor spot) on the surface of the host cell (13, 15, 23). This is the same mechanism as that postulated for the equally specific antigen-antibody reaction. In the latter case the forces holding the two together are supposed to be of the exchange or van der Waals type and hence fall off so rapidly, as the distance between the surfaces increases, that they become negligible for separations of a fraction of an Angstrom unit (60). If such surfaces are rigid but possess a degree of complexity which is compatible with the degree of specificity observed, one might expect that the chances of their accidentally colliding in just that relative attitude which leads to adhesion would be very small indeed. One would expect a steric factor in the reaction rate expression which would be inversely related to the complexities of the surface or the specificity of the reaction.

Since a bacterial cell is capable of supporting the growth of 100 or so different viruses, it is supposed that its surface contains a corresponding number of *different* types of receptor spots, one for each type of virus to which it is sensitive. It is also estimated that a cell can adsorb on its surface about 140 virus particles of any one type (66); it would therefore be supposed to possess a corresponding number of receptor spots for each of the viruses to which it is sensitive. Mutation of the host to resistance to a particular virus would thus imply the removal of all the receptor spots for that virus, since bacterial cells do not adsorb the viruses to which they are resistant (50).

On such a simple theory of adsorption one would expect that the chances of a particle sticking to the host in any random collision would be low by a factor of at least a thousand to account for the specificity of the reaction, and to be low by another factor of 100 or so to take account of the fraction of the host's surface which is occupied by the particular receptor spots for the virus. But the calculations presented in the previous section suggest that the virus adheres to the host almost every time the two collide. Obviously the simple receptor spot theory presented above is in difficulty here. Before discussing possible modifications of the theory, we shall turn first to a discussion of some more recent data regarding adsorption.

Effects of Stirring. It might be thought that the collision rate and hence adsorption would be accelerated by violent stirring of the mixture of virus and host cells. However, it turns out that T_4 virus in an ammonium lactate medium containing 0.02 mg. of *l*-tryptophane per cc. for activation (see following section) is not adsorbed on the host in a Waring type blender. As soon as the blender is turned off, however, the virus is adsorbed at the normal rate; the adsorption may again be stopped by turning on the blender (unpublished results of the author). Neither the virus particles nor the host cells appear to be damaged by this treatment under the conditions employed (5×10^9 B/cc.; 2×10^7 T_4/cc.; temperature, 26°). It seems probable from these results that the primary contacts between virus and host lead to relatively weak initial bonds between *flexible* areas on the two surfaces which then rapidly conjugate like the two parts of a zipper. The shearing forces in the blender are presumed to break the weak initial bonds before the more extensive contacts are formed. The experimental results suggest that the time required for the pair to form a firm bond may be ten seconds or less. More precise experiments of this type are certainly feasible and should be carried out.

Virus Co-factors (5, 7). The major fraction of some phage preparations is scarcely adsorbed at all in synthetic media, while adsorption is normal in a complete medium. This phenomenon has the aspects of a nutritional requirement of the virus. Many preparations of T_4 and T_6, for example, are found to have very low titers from plaque counts on agar containing ammonium lactate (F) as nutrient as compared to those on agar containing a more complete nutrient such as Difco nutrient broth (N). On the other hand, both media supported voluminous growth of the host bacteria. Tests of the activities of amino acids and other growth factors showed that aromatic amino acids, such as *l*-tryptophane, phenylalanine, tyrosine and many synthetic amino acids, would promote the *adsorption* of the phage in the synthetic medium. Among the substances is 5-methyl tryptophane, a compound whose bacteriostatic action on *E. coli* is reversed by *l*-tryptophane (6).

The co-factor is not required for multiplication of the virus within the cell, for once a T_4 particle has become adsorbed on a host cell, the infected cell is able to form a plaque at 37° on the synthetic medium. Thus T_4 must have been able not only to multiply in the cell and lyse it, but carry the infection to other cells on the smear as though enough tryptophane or some other co-factor were present to keep the newly formed virus in an active state. The activity of *l*-tryptophane is negligible at about 0.0001 mg./cc. and reaches its maximum value at concentrations of only 0.002 mg./cc. or so.

It has been found that when plaques of T_4 which appear on agar containing the synthetic medium are picked up and suspended in synthetic medium, the suspension assays almost as high on F agar as it does on N agar. On the other hand, suspensions of plaques which appear on co-factor-free synthetic medium from bacteria which have been infected by tryptophane-activated virus, yield much lower assays on F agar than on N agar. This result indicates that the requirement or non-requirement for co-factor is inherited in the virus T_4(*7b*). Evidence for mutation from requirement to non-requirement comes from the observation that all tested stocks made from isolations of single plaques of the co-factor-requiring type contain variable amounts of the non-requiring type of virus. Mutations in the reverse direction, *i.e.*, from the non-requiring to the requiring type, have not yet been looked for.

Tryptophane acts on the virus rather than on the host; for if to virus in 0.1 cc. of the synthetic medium containing tryptophane at 0.002 mg./cc. are added 10 cc. of a bacterial suspension, suddenly bringing the tryptophane concentration down to the ineffective level of 0.00002 mg./cc, as much as 15% of the virus may be adsorbed. On the other hand, adding an equivalent amount of tryptophane to a mixture of B and T_4 results in the adsorption of no more than 0.001% of the virus particles.

The effect of the tryptophane on the virus is reversible, for on removal of tryptophane from the virus by dilution, for example, the virus loses the ability to attack the host cell. Such deactivated virus can be reactivated by addition of more tryptophane. *d*-tryptophane is inactive. As in many enzyme reactions, the activation is maximum at 37°, and at pH around 7. In this connection it is interesting to note that the efficiency of plaque formation on F by B infected with T_4 and T_6 falls to a low value at 15° as compared to 37° in agreement with the idea mentioned previously that some bacterial product, perhaps tryptophane, is responsible for the activation of the freshly liberated virus in the chain of infections which lead to the formation of a visible plaque.

Of the other viruses in the T set only T_7 exhibits such a temperature dependence of plaque-formation on F agar, but not on N agar. The efficiency of plaque formation by T_7 at 15° can be brought to the value on N agar by the addition of leucine, isoleucine or norleucine to the F agar. Such compounds may therefore be co-factors for T_7, being supplied in sufficient quantity by the bacterial metabolism at 37° to activate this virus (unpublished results of the author).

These results are compatible with the idea that the inactive virus particle T_4 must take onto itself a number of molecules, *n*, say, of tryptophane to form an activated virus particle, T_4^*, before it is capable of being attached to the host cell. The reaction might be written as a reversible equilibrium:

$$T_4 + n\ l\text{-tryptophane} \rightleftarrows T_4^* \qquad (7)$$

If the *n*-tryptophane molecules occupy *equivalent* non-interacting sites on the virus, the equilibrium constant would be:

$$K = \frac{(T_4^*)}{(T_4)\ (l\text{-tryptophane})^n} \qquad (8)$$

On this basis one may estimate the value of *n* from the number of infected host cells which are produced when 10 cc. of a bacterial suspension are added with moderate stirring to 0.1 cc. samples of T_4 in the synthetic medium containing various amounts of *l*-tryptophane. Values of *n* as high as 6 have been obtained in this way, although the true value may be much greater than this, for all the virus particles in the suspensions tested may not have had identical requirements for tryptophane.

It should be possible to obtain evidence for the number of tryptophane molecules required for activation by a study of the dependence of the rate of activation on the tryptophane concentration. This has not yet been done. However, it has been found that the rate is so rapid in F containing 0.002 mg. *l*-tryptophane/cc., that equilibrium is approached in a few minutes at 26°.

The rate at which T_4^* particles *lose* their activation on reduction of the tryptophane concentration to below the critical level (0.0002 mg./cc.) has been studied in some detail and by a number of different methods. The reaction yielding deactivated virus T_4'

$$T_4^* \xrightarrow{K_2} T_4' + \text{tryptophane (?)} \qquad (9)$$

seems to be first order, since it follows the relation:

$$-2.3 \log \frac{(T_4^*)}{(T_4^*)_0} = K_2t \qquad (10)$$

where $(T_4^*)_0$ is the concentration of active virus at time $t = 0$. This result suggests that the loss of only one of the *n* *l*-tryptophane molecules required for activation leads to deactivation and the loss of the ability to attack the host.

Mutation Pattern of B to Resistance. If the host (B) mutates to become resistant to one of the viruses in the T set, it frequently becomes resistant to certain of the other viruses at the same time. Resistance to certain sets of viruses appears to be linked together and frequently involves new growth requirements as well. Thus the 377 resistant strains tested by Demerec and Fano (32) consisted of only eight types: B/1, B/1,5, B/3,4, B/3,4,7, B/6, B/1,3,4,5, B/6,1,5 and B/7,1,3,4,5. A few more types involving resistance to host range mutants of T_1 and T_7 have been added by Luria (48–50). An analysis of the mutation rates shows that these are all "one step" mutations. It is probable that sensitivity to each phage should be considered in principle to be an independent phenotypic character, but, as has been frequently observed in higher organisms, more than one character may be affected by a single genetic change. Such theoretical aspects of mutations to virus resistance have already been discussed in very able fashion (1, 2, 29, 49–51). The point to be emphasized here is that these mutations affect the adsorption mechanisms for the viruses involved. These so-called "cross-resistance" tests do not relate the viruses. If anything they relate receptor mechanisms for different viruses. A single genic locus may be considered to control the production of a group of receptor mechanisms for a corresponding group of viruses. A non-lethal mutation of such a locus could then eliminate the entire group of receptor mechanisms and still allow the bacterium to survive and multiply to form a clone of resistant cells.

Discussion of the Mechanism of Adsorption. Any theory of the mechanism of adsorption must be compatible with the following facts:

a. A phage is adsorbed only on cells which under suitable conditions are capable of supporting the multiplication of that phage. The reaction is, therefore, highly specific.

b. In quiet media a virus particle has a good chance of being adsorbed on the host the first time it approaches the surface of the cell.

c. In violently agitated media T_4 virus is not bound to the host cell, although the rate of collision between the two must be increased by agitation.

d. There are strains of T_4 and T_6 virus which are not adsorbed on sensitive cells unless they have been activated by a co-factor such as *l*-tryptophane.

Neglecting for the present the possibility of long range forces between the phage and host (63, 58), we must assume that adsorption involves material contact between elements of the host and parasite. Somehow the element of specificity must enter, whether in steric configuration or in enzymatic sequence or both. The rapidity of the reaction in quiet media suggests that the initial contact may occur between small specific projecting elements on the host or on the virus. Such elements would have high thermal velocities (Brownian motions) relative to those of the larger bodies to which they are attached. During the time the virus and host are near each other in diffusing through the medium, such mobile elements would make many collisions; one collision in which the components are properly oriented would lead to the steric fitting of the elements and the formation of a weak bond between virus and host. Such a tentative bond can be broken by stirring, but in quiet media holds the two bodies together long enough for some subsequent chain(s) of reactions to lead to more firm bonds between the two. It may well be that the specificity of the combination may reside in the zipper-like sequence which leads to this ultimately firm combination. The weaker combination would be broken in a few seconds by thermal agitation and therefore might not be expected to be detected in ordinary experiments carried out by manual operations of solutions in test tubes, pipettes, *etc.*

The co-factor phenomenon is still a puzzling clue to the adsorption mechanism. The activity of *l*- and inactivity of *d*-tryptophane and the characteristic temperature dependence of activation both suggest that enzymes may enter into the reaction. The involvement of a number of molecules of tryptophane suggests deficiencies on a number of sites on the virus; but the loss of one of the molecules results in deactivation and no firm adsorption. Apparently tryptophane would be essential for each of a number of the postulated steps in the adsorption reaction. These steps may involve syntheses of material containing tryptophane or, alternately, containing the other aromatic amino acids which are co-factors.

The receptor mechanism appears to occupy a key position in any theory of virus activity. If we restate the generally accepted experimental observation and the postulate, the conclusion seems inevitable:

a. A phage is adsorbed on only those cells which, if not damaged (see *Contributions of the Host*), are capable of supporting its multiplication.

b. The ability of a cell to adsorb a virus is determined by the presence on that cell of a receptor mechanism specific for that virus.

c. Therefore mutation of the host to resistance *and resistance to viruses in general,* if we accept the doctrine that evolution results from mutation, involve only alteration or elimination of the receptor mechanism for that virus. It would follow that any cell, as long as it displays the receptor mechanism for a particular virus, is a suitable host for that virus.

What, then, could be the nature of the receptor mechanism? Is it simply a code system on the host structure fashioned to the virus but unrelated in any direct manner to the metabolic patterns contained within? This seems to be an unlikely and awkward arrangament, for resistance.seems to be intimately related to metabolic patterns of the host in the cases which have been studied (1, 2). It seems much more likely that in the receptor mechanism is incorporated a frank display by the host of its metabolic suitability for the multiplication of the viruses to which it is sensitive.

Burnet (12) and others (15, 36, 37) (but see (9)) have concluded that in bacteria the surface antigenic polysaccharides are important constituents of the receptive mechanism, for they found that cell-free extracts containing these substances have the power to combine with and inactivate phages which are active on the original cell. Furthermore in a number of cases susceptibility to phage action is correlated with "smooth" or "rough" forms, the colony type being determined by the presence or absence of surface polysaccharides.

A further clue is provided by Mrs. Pirie's observation (61) that exposure of *B. megatherium* to lysozyme strips mucopolysaccharide from the cells and simultaneously renders them non-receptive to the phage to which they had been sensitive before treatment with the enzyme. Recently Burnet and his co-workers (16, 70) have made a somewhat similar observation in the case of influenza virus. They have discovered that a culture filtrate of *V. cholerae* has the power of rendering red cells nonagglutinable by the virus. Likewise the membranes of the chick embryo do not adsorb virus hemagglutinins after treatment with the extract. Behaving much like the virus, the active principle in the filtrate is adsorbed by red cells and later eluted. It appears that the bacterial filtrate contains an enzyme which, like the virus, can destroy the receptor mechanism of the red cells. The substrate of the virus-enzyme seems to be a segment of the mucin which is responsible for the A B O blood group specificity (15*a*).

How can such substances as surface polysaccharides and mucopolysaccharides display the finer points of the internal constituents of the cell? Solution of this problem would contribute greatly to our understanding of the mechanisms involved in the biosynthesis and function of these constituents.

Adsorption represents the first step in the conjugation of virus elements with host elements to form a complex which, under suitable conditions of temperature, nutrition, *etc.*, forms more virus. However, mere adsorption of virus on the host cell is not sufficient for virus production. Damaged virus may be adsorbed on the host, yet no virus particles will be liberated. Similarly intact virus adsorbed on sufficiently damaged host cells is unable to induce the formation of more virus. We shall now discuss the nature of these additional elements of the two which go to make the resulting complex functional, *i.e.*, able to manufacture new virus.

BIOLOGICAL COMPOSITION OF THE VIRUS-HOST COMPLEX

In the T system of viruses the combination of a virus particle with its host cell is apparently indissoluble. The virus-host complex has the form of the host, but the viable host has never been recovered from it. It has the function of forming new particles similar to the infecting virus particle; yet the infecting particle has never been freed from it before lysis. We must inquire: What constituents are necessary for a properly functioning complex, *i.e.,* one which will produce virus? Obviously the constituents of the virus-producing complex are derived from at least two sources: the virus and the host cell. The co-factor phenomenon indicates that elements of the medium also play a role.

Contributions of the Host

We have already discussed the primary contribution in point of time of the host to the virus-host complex—the adsorption mechanism. Mutations of the host to resistance involve at least the elimination of that mechanism for the virus in question but may eventually be found to involve other constitutional changes such as nutritional deficiencies (1, 2). But the host contributes much more than the adsorption mechanism, for it may be treated with heat, chemicals, irradiation or indeed with another virus and be left with an apparently intact ability to adsorb new virus and yet be rendered completely incapable of forming a complex which can produce that virus. On the other hand, some agents such as dilute antibacterial serum (26), merely delay the liberation of virus from the infected bacteria.

Effects of Irradiation of Host Cells. It has been found (3) that T_2 virus can multiply on host cells which have received many lethal doses of ultraviolet irradiation. Likewise phage C16 can multiply on *"bacille paradysenterique Y6R"* which has previously been heavily irradiated and "killed" with X-rays (64). We may conclude from these results that *all* the elements of material and organization which the host requires for its continued multiplication to form colonies are not necessary for the formation of a functional virus-host complex.

Many lethal doses of irradiation of B are required to make the cells unsuitable as hosts for T_2 (3). With massive doses two effects appear at approximately the same exposure: both the proportion of active virus-producing complexes formed after adsorption and the number of active virus particles liberated per active complex decrease as the pre-infection exposure is increased. Still more severe irradiation of B eventually reduces the ability of the cells to adsorb T_2. The fact that the two effects are produced by irradiation at the same rate may be a coincidence or it may be an indication that similar host elements are involved in the two phenomena. The elements with which the virus first combines on adsorption may be just those involved in virus production in a chain reaction of some sort within the complex. Much as analogues of normal substrates are presumed to inactivate enzyme molecules which they engage, so the UV-produced structural analogues of the host's elements may be presumed to inactivate elements of the virus which they engage.

Killing of the Host by Damaged Virus Particles. The converse relation also appears to hold, namely, a single T_2 virus particle partially inactivated by UV has the property of combining with and killing host cells (54). An individual inactivated virus cannot form a functional complex, *i.e.,* produce new active virus; nor can the inactive complex form a colony of bacteria. The combination is sterile. Apparently such inactivated virus combines with elements of the host which are necessary for its continued multiplication to form a colony.

Studies of the respiration of rapidly growing cultures of B in ammonium lactate medium show that infection with normal T_4, normal T_2 or T_2 virus which has been partially inactivated by irradiation causes the rate of oxygen uptake to remain constant at the value the culture had at the time of infection (19); uninfected control cultures of course continued to increase logarithmically both in number of cells and in rate of oxygen consumption. Thus the viruses did not interfere with oxygen *utilization,* but apparently blocked *synthesis* of the lactic acid dehydrogenase system of the infected cells. This blocking of synthesis of host material is strikingly brought out by recent experiments of Monod and Wollman (57 *a*). They found that normal *E. coli* B, using glucose as a carbon source, could readily adapt its metabolism to the utilization of lactose. However, after infection with a phage, designated ϕ II, it is rendered incapable of adapting its metabolism to the utilization of lactose, presumably because it cannot synthesize the necessary new enzymes. As discussed later, the syntheses which the virus-host complex carries out during its development are directed by the infecting virus rather than by the regulating mechanisms of the host. The synthesis-directing mechanisms of the host play little part in the development of the complex; their functions are taken over by the virus.

We conclude from these scanty experiments that all the elements required for the host's well-being are not required for the formation of the virus-producing complex. On the other hand, some of the elements (*e.g.*, synthetic) essential to the host seem to be engaged in the formation of the complex. The nature of these elements is only weakly suggested by their relatively low sensitivities to irradiation relative to those of either the host's or virus's survival. However, this circumstance has made possible the interpretation of experiments involving irradiation of the developing virus-host complex to be discussed later.

Interference Between Unrelated Viruses. As far as we now know, the infection of a cell with two viruses which are unrelated to each other morphologically or serologically results in the formation of a complex which is capable of liberating either one or the other virus but never both, for when such mixed complexes are plated with mixtures of strains of the host (one resistant to one virus, the other resistant to the other), the plaques obtained are always cloudy. This indicates that one of the indicator strains has grown, *i.e.*, that *both* viruses have not been liberated (*27*, *29*, 31). Since infection by one of the viruses interferes with production of the other, this phenomenon has been termed the "interference" phenomenon. As we shall see, the more detailed studies of this phenomenon suggest that two viruses may compete for elements of the host which are essential to both.

When a cell is infected simultaneously with T_1 and T_2 the complexes formed are capable of yielding only T_2. Moreover, the "latent period," or time interval between infection and lysis, is 21 minutes, the time characteristic of lysis by T_2 rather than 13 minutes which is characteristic of lysis by T_1 (31). T_2 may be said to be "dominant" over T_1. Similarly T_7 is slightly dominant over T_1. T_2 is dominant over T_7, so the three may be arranged in a series of dominance $T_2 > T_7 > T_1$ (*27*). It would be interesting if other viruses in the T set could be placed unambiguously in such a series of dominance with unrelated strains.

The dominance of one virus over another can be overcome by infecting the host with the latter virus before exposing it to the first. For example, if T_1 is given a five minute "head start" over T_2, most of the complexes liberate T_1. These data first suggested that the several viruses competed for a single "key enzyme" molecule present in the host and necessary for the multiplication of viruses. The virus, in mixed infection, which first engaged the "key enzyme" molecule was thus supposed to interfere with the others (31). The interval between adsorption and capture of the key enzyme would determine a virus' position in a dominance series.

Extended studies of interference between T_1, T_2 and T_7 has shown, however, that the virus whose multiplication is blocked can decrease the yield or burst size of the successful one. This "depressor effect" diminishes as the interval between the adsorption of the two viruses increases, and vanishes at a time which is characteristic of the strain of virus that is eventually liberated from the cell in question (27).

Further data came from experiments using antisera against the competing viruses. A virus host-complex is immune to antisera to the virus. This is taken to indicate that antibodies cannot penetrate the infected cell, although the antigenic identity of "intracellular virus" and free virus particles, with which antisera are made, has not been demonstrated. Now the virus yield in mixed T_1, T_7 infection was observed to be increased by antiserum to the depressor virus added *after* its adsorption. It thus appeared that the depressor virus could lie only on the bacterial surface where it would be subject to attack and inactivation by its antiserum (27). As in the exclusion of all but one sperm in the fertilization of monospermic eggs, the unsuccessful virus was pictured as being excluded from the cell by a membrane which spread rapidly over the cell and attached daughter cells, too, soon after entry of the first particle. Even here, however, it was considered to be able to compete with the successful virus for some substrate made by the cell and required by both, thereby depressing the multiplication of the intracellular particle.

Certainly the depressor effect suggests competition between viruses. The interference phenomenon might well be the result of such competition for a number of host elements or enzymes, one virus almost always monopolizing the supply in a series of unexpectedly rapid reactions following adsorption. The work on mixed infection was begun in the hope of inducing host cells to lyse through the action of one of the viruses (*e.g.*, T_1, whose latent period is only 13 min.) before the other virus (T_2, latent period 21 min.) could have done so, thus revealing intermediate stages in the "multiplication" of the slower virus. Until now the techniques used in studies of interference could detect only fully active virus. The phenomenon should be reexamined with electron microscopic and chemical techniques which might reveal the presence of intermediate non-infectious stages in the development of the complex between the unsuccessful virus and the host constituents.

Contributions of the Virus

Here again much of the data comes from experiments in which ultraviolet irradiation has been used as a means of destroying elements of the virus particle, and reducing its ability to form an active virus-producing complex after combining with the host. The results obtained depend in a striking fashion on whether the complex is formed from the combination of the host with one or with more than one virus particle, and whether they are of the same or of different type.

Single Infection. In studying the inactivation of a virus by ultraviolet, one ordinarily irradiates samples of the virus with various doses, and then dilutes the suspensions in nutrient medium before making up the adsorption mixtures with the host cells for assay by the plaque-count method. By the procedure the ratio of virus particles to bacteria is 0.001 or less, and the fraction of cells having more than one virus particle adsorbed on it as a consequence will be less than 0.000001 (Eq. 1). Under such conditions of single infection it is found that the fraction, y, of virus T_2 or T_7 which can form active virus-producing complexes decreases with the doses of irradiation, D, exactly according to the "one hit" relation:

$$y = e^{-aD}. \tag{11}$$

Here a is a constant characteristic of the virus and the irradiating wave length (46, 57).

The virus which survives is somehow altered by the irradiation (47). The virus-host complexes $B:T_1$, $B:T_2$ and $B:T_7$ formed with survivors exhibit lysis delayed by as much as 18, 3 and 15 min., respectively. The burst sizes, however, are normal, and the virus produced is normal. The effect on the virus is "physiological" rather than hereditary, and demonstrates that some elements of the virus may be altered by irradiation without destroying its "viability". It would be important to know whether these effects are due to a breaking of chemical bonds, to the formation of stable structures isomeric with the intact virus, or to the storage of vibrational or electronic energy in the virus particles. In connection with the latter possibilities it was noted that the irradiated virus did not "recover" its rate of lysis after incubation for a day at 5°.

Irradiation of either $B:T_2$ or $B/r:T_2$ (B/r is the UV-resistant strain of B (73) immediately after their formation gives one-hit curves for destruction of the virus-producing complex (57). The rate constant, a, is reduced only slightly below the value for free virus, presumably by the partial shielding which the less sensitive cellular constituents afford the virus elements. Similar results are obtained with $B:T_7$ and $B/r:T_7$. An interpretation of these killing effects comes from recent studies of multiply infected cells.

Multiple Infection. The irradiation of $B:(T_2)_m$, freshly formed by the combination of a number, m, of T_2 particles with a single host cell, yields (57) "multiple hit" curves for its survival as a producer of virus:

$$y = 1 - (1 - e^{-aD})^n \qquad (12)$$

The theoretical number of hits, n, corresponded rather closely to the multiplicity of infection, m. This would be the expected result if each of the adsorbed particles were able to contribute independently to the formation of a virus-producing complex. However, in multiple infection the apparent sensitivity (a) of the adsorbed particles turned out to be much smaller than might have been anticipated from the results of irradiation of singly infected cells. The questions raised by these puzzling observations seem to be answered by Luria's recent important studies on the effects of *multiple* infection with virus which has been inactivated by ultraviolet irradiation.

He finds (52) that if irradiated T_2 suspensions are assayed under conditions in which an appreciable number of the bacteria used for assay have adsorbed *more than one* partially inactivated particle, the number of active virus-producing complexes formed is much greater than would be formed if the bacteria have been singly infected. Indeed, the number of active complexes formed by T_2 particles which have received three or four lethal doses ($a\,D = 3$ to 4 in. Eq. 11 as estimated by single infection assays) is equal within experimental error to the number of host cells which have adsorbed two or more virus particles. It is as though an inactivated T_2 particle adsorbed on the host cell could be reactivated by another inactivated T_2 particle adsorbed on the same host cell. Pairs of T_2 particles which have received progressively larger doses of irradiation show decreasing capacities for producing active virus when adsorbed on the same host cell. These phenomena are exhibited by the phages T_2, T_4 and T_6 as well as by T_5, but not by T_1, T_3 or T_7. Inactivated even-numbered viruses can be reactivated by other even-numbered serologically and morphologically related viruses, but not by T_5 or by the other odd-numbered unrelated viruses.

By analogy with the observed (29, 41) transfer of genetic characters between phage particles growing in the same host cell (see Genetics of Viruses), these data suggest that transfers between elements which have been destroyed by irradiation can occur in the virus-host complex. A single such "lethal" in any phage particle renders it incapable of forming an active virus-host complex by single infection, for in it at least one element of the set of constituents which it requires to be active has been destroyed. Hence the "one hit" curve for single infection referred to in the previous section. The missing elements can be supplied by a second virus particle if none of the alleles of the missing elements has been destroyed by the irradiation of the second particle. On this basis the number of such essential elements can be estimated. A complete set must contain considerably more than three elements, since three hits per particle hardly reduce the chances for *pairs* of particles to produce active virus-host complexes in combining with a single host cell. An estimate of the number has been made from a statistical analysis of the decreased efficiency with which pairs of virus particles which have received five or more hits can complete each others sets of damaged loci. The analyses indicate that the even-numbered viruses must have between 35 and 50 essential loci, in each of which lethal mutations of this sort can be produced by ultraviolet irradiation.

Incidentally the fact that irradiated even-numbered phages can reactive each other after adsorption on the host indicates that they can complete each others sets of loci; these related viruses possess allelic elements. Although not yet proven, it seems likely that these elements are those which determine inheritance in these viruses.

Lysis Inhibition. We now turn to a rather odd phenomenon observed when certain strains of virus are adsorbed on a virus-host complex rather late in its development (33). Stocks of T_2 T_4 and T_6 contain two types of phage. One, designated by the symbol "r", produces rapid lysis of broth cultures of B and gives clear plaques. The other, designated by "r^+", usually predominates—hence the "+" to designate "wild type" in accordance with genetic notation in other forms. The latter type gives visible lysis of turbid broth cultures delayed by five hours or so. On the other hand, the two strains, r and r^+, lyse highly diluted broth cultures at the same times after infection.

Inhibition of lysis has been shown to be produced by the T_2r^+, T_4r^+ or T_6r^+ phage particles themselves; liberated from a few phage-producing centers when the bacteria begin to lyse, they prevent lysis when they are adsorbed on the remaining centers which are about to lyse. The r strains do not do this. Apparently the addition of some virus constituents present in the r^+ particles to complexes of related virus strains has the effect of delaying lysis of such complexes. The mechanism is still obscure.

GENETICS OF VIRUSES

Spontaneous Mutation. When a bacterial virus is assayed by counting plaques produced by singly infected host cells, each plaque contains between 10^7 and 10^9 virus particles, members of a single clone of virus particles. Usually the majority of the particles in a clone are phenotypically similar and presumably possess the characters of the original particle. On closer examination of such clones, however, it is found that a small portion of the particles differs phenotypically from the majority.

Thus Hershey finds (41) that r plaques arising from a strain of T_2, designated T_2Hhr^+, contain about 0.1% of virus particles which produce r plaques and are therefore designated T_2Hhr. Evidence for the reverse transition has not been discovered by the analysis of simple r plaques. However, after serial passage on broth cultures of B T_2Hhr, stocks always revert to the r^+ type. Apparently the transition $r \rightarrow r^+$ occurs with a much lower frequency ($\sim 10^{-8}$) than the reverse, $r^+ \rightarrow r$ ($\sim 10^{-8}$);

but in broth, at least, the r^+ has a selective advantage over the r type and outgrows it. Such strains of r^+ recovered from the reverse transition revert to r with the same apparent frequency as the original parent strain. This suggests that the $r \rightarrow r^+$ is truly the reverse of the original mutation $r^+ \rightarrow r$.

After more experience with the material, Hershey was able to observe reversible phenotypic mutations to four *degrees* of lysis inhibition. The mutation pattern which he observed is understandable in terms of three independently mutable factors, each contributing to lysis inhibition. The most frequent mutations he observes are in all cases those leading to *less* lysis inhibition. In spite of this, the non-inhibiting clones invariably revert to wild type after extensive serial passage. No obvious changes in host range are connected with these mutations.

Luria first studied host range mutations of T_1 and T_2 (48). A large series of individual T_1 and T_2 cultures, started by the action of only a few virus particles growing on B in broth, contained after incubation an average of about one T_1' particle or T_2' particle (active on B/1 or B/2, respectively) in 10^7. Both the T_1 and T_2 cultures contained extremely variable proportions of the mutant, however, evidence that the changes were true mutations which occurred at random in the growth of the cultures rather than qualitative changes affecting equal proportions of virus in the separate cultures. Hershey obtains (41) similar results with host-range mutations in single clones of T_2H and has shown that at least some of them appear to be truly *reversible*. Moreover, host range mutations and the plaque type mutations occur independently in his material.

As our acquaintance with these viruses becomes broader it will be possible to recognize more types of phenotypic character. For example, the co-factor requirement of wild type T_4 and T_6 is lost by mutation to types which do not require added co-factor (T. F. Anderson, unpublished).

Since such alterations in character have so far not been detected as a result of treatment of virus *particles* with UV, X-rays or heat, it is supposed that they arise through aberrations in the biosynthetic pattern. The mechanism of the biosynthetic processes is unknown; such aberrations in these patterns are therefore termed "spontaneous mutations".

True estimates of the frequency of spontaneous mutations in viruses, as in other biological systems, will have to await an understanding of the mechanisms of virus and gene production. Even then the data on large clones will be ambiguous unless we understand how such factors as multiple infection and selection influence the proportion of each of the types in the population chosen for analysis. For example, in the young clones of phages those types having higher rates of adsorption and shorter times of lysis will tend to be favored. In the old clone, however, since the particles adsorbed on developed virus-host complexes may be inactivated, the types with higher rates of adsorption may tend to be eliminated as the clone exhausts the available uninfected host cells. Multiple infection may influence some mutation rates. If so, old clones such as plaques or lysates will be difficult to analyze. Many of the particles in such a clone doubtless arise from multiply-infected cells: on agar, those adjacent to the spreading plaque; in heavy broth suspensions, the last of the bacteria to lyse will be multiply-infected. These difficulties are well realized by workers in the field. Those involving population dynamics can be overcome. The mechanism of gene production is the crux of many biological problems.

The advantages of virus material for the study of mutation are obvious to workers in the field. The cheapness, ease and rapidity with which huge clones can be grown and analyzed are factors which, together with the relative chemical simplicity of virus material, justify the hope that inheritance will eventually be explainable in the language of physical chemistry. On the biological level, at least, much of our information will come from studies of mixedly infected bacteria to which we now turn.

Exchange of Characters between Related Viruses. Early in 1945 (*cf.* 30) Hershey discovered that mixed infection of B with T_2r^+ and T_2r gave rise to mottled plaques which he showed contained both type of virus. Apparently, mixed infection of single host cells had produced complexes which formed both kinds of virus. Since then both he (40, 41) and Delbruck and Bailey (30) found that many bacteria, multiply-infected with pairs having host range markers (T_2, T_4), (T_4, T_6), (T_2, T_6), produce both of the infecting types. "Mutual exclusion" breaks down more frequently in pairs involving T_2, less frequently in those involving the pair (T_4, T_6) (30).

A still more interesting and important phenomenon is observed when more than one pair of phenotypic markers is used in the infecting pair (30, 41). Thus infection with the pair (T_4r, T_6r^+) yields, besides the infecting pair, also the viruses (T_4r^+ and T_6r). Similar phenomena occur in other pairs of viruses. Delbruck speaks of these as "induced mutations"; not only does T_4r "induce" T_6r^+ to become T_6r, but T_6r^+ "induces" T_4r to become T_4r^+. The nature of the phenomenon can better be appreciated from experiments involving more than four phenotypic markers.

Hershey (41) has studied the populations in plaques produced by B infected by the pair (T_2h^+r, $T_2h^bh^cr+$), each of which breeds true (h^b and h^c refer to altered host range from wild type h^+). All but one ($T_2h^br^+$) of the eight possible combinations of the characters were found in the population. How much reshuffling of the characters occurred in the parent complex, and what part selection played in determining the population figures given, will have to come from further studies, *e.g.,* of single bursts. The variety of hybrids which Hershey obtains is strong evidence that genic factors in viruses are separable. It seems that this great number of possible combinations of such genic factors "serve (s) in nature to multiply the seemingly unlimited array of existing types. . . . of viruses" (41).

Recently Hershey and Rotman (41*a*) have extended these studies by examining crosses between some 15 independently arising r mutants designated T2Hr1 to T2Hr15. Each mutant grown by itself bred true, producing wild type, r^+, very infrequently. The progeny of mixed infections after one-step growth all contained relatively large proportions of wild type, indicating that exchanges of genic character between types occurred and that the mutants were all different. The crosses fell into two classes on the basis of the percentages of wild type they produced, suggesting that there are two independent linkage groups of r loci. "The existence of some kind of linkage system conditioning segregation and reassortment of genetic factors among viral particles seems quite clear, but it remains to be learned whether reciprocal or even material exchanges are involved".

DEVELOPMENT OF THE VIRUS-HOST COMPLEX

So far we have discussed the virus particle, its adsorption on the host and the manner in which the biological composition of the resulting complex affects the virus products which are ultimately formed. It was always evident that some process occurred in the infected host to cause it to liberate new virus particles, but until recently no experimental evidence of the nature of these processes was available. Recently experimental proof of the changing nature of the complex has been provided by many lines of investigation. As yet the confusing multitude of clues gives only a vague picture of the developmental processes.

Effects of Radiation on the Developing Complex. We have already seen that two minutes after their formation, B:T_2 and B:T_7 complexes are only slightly less sensitive to ultraviolet irradiation than the free virus particles themselves. For both free virus and freshly formed complexes one-hit inactivation curves are obtained. However, starting five minutes after its formation the sensitivity of a suspension of B:T_2 falls off rapidly until 12 minutes after infection the rate of inactivation is only about one-fifth its initial value (57). As the complexes develop still further, however, their average sensitivity rises two or three-fold again until lysis occurs at 21 minutes.

The initial decrease in sensitivity could easily be accounted for by assuming an increase during development in the number of phage-producing units, the number varying widely between individual B:T_2's about a mean value in accordance with the variations which are observed in individual burst sizes. It is the unexpected later *increase* in sensitivity which makes the data impossible to interpret without an additional postulate. In this connection it seems most significant that for multiple infection the development reaches in only six minutes the stage of minimum sensitivity, which is reached in 12 minutes in single infection. Still the *time* of lysis and average burst size are reported to be the same in single and multiple infection (24, 31).

Luria and Latarjet (57) see the initial decrease in sensitivity as being possibly caused by an accumulation of absorbing material around the phage particle within the host. The subsequent increase in sensitivity is taken to indicate "that, as phage multiplication proceeds, the apparent sensitivity of the intracellular phage particles returns to higher values".

Using X-rays to inactivate growing intracellular T_2, Latarjet (45*a*) finds that for seven minutes after its formation the resistance of the complex is equal to that of the free veins, then from seven to nine minutes the resistance increases. Starting at ten minutes multiple-hit curves are obtained, increasing in multiplicity until 13 minutes when an average multiplicity of 130 is obtained but with a resistance approximately twice that of the free virus particle. From 13 minutes until lysis at 21 minutes the curves retain their multiple-hit character, but the resistance to X-rays slowly decreases to that of the free particle.

Clear cut evidence for the formation of intracellular virus has been obtained by Doermann (33a) who has lysed B : T_4 at intervals after infection, using an excess of T_6 and KCN for this purpose. Complexes lysed by this technique liberate no infectious T_4 particles until about 14 minutes after infection when an average of one per complex is found. The number of T_4 particles liberated then increases linearly until the normal burst size is reached in 28 minutes.

Similar results have been obtained by the use of sonic vibration to disrupt the infected cells. Since the phages T_2,T_4,T_5 and T_6 are rapidly inactivated by sonic vibration, this method is limited to the small phages T_1, T_3 and T_7 which are resistant (7d). B :T_3 was found by either the sonic or KCN-T_6 method not to contain infectious T_3 particles until some 13 minutes after its formation (under the experimental conditions employed). A rapid increase in T_3 liberated per complex then occurred (well before normal lysis commenced at 18 minutes), and by 25 minutes had reached the number liberated by normal lysis.

From the results outlined in this section it appears that in infecting a host cell the incoming particle retains its X-ray sensitive volume for some time, but in this period it changes its state by combining with host material or otherwise, so that it can neither produce daughter particles nor infect other host cells after its complex with the host cell has been disrupted. Eventually in the intact complex this form apparently establishes regions equal in number to the burst size at which virus particles are later assembled. These regions have roughly half the sensitivity to X-rays exhibited by the free virus and are incapable of completing virus particles when the cell is lysed prematurely. Within the complex, however, completed virus particles are assembled at these regions, and these may be liberated by either normal or artificial lysis of the complex. As will be discussed in a later section, the completion of the even-numbered viruses correspond in time at least to the synthesis of desoxypentose nucleic acid.

Variations in Light Scattering. The variation in turbidities (33) of B:T_4r^+ and B:T_4r suspensions clearly indicates that rather extensive changes occur during the development of the complex. Immediately after five-fold infection the light scattering from an actively growing culture of B in broth begins to drop and in a few minutes reaches a minimum 20% below the initial value. The scattering power then rises to 95% of its initial value just before lysis.

The author has observed (4) a similarly abrupt 40-60% decrease in opacity of a heavily UV-irradiated culture of B grown in ammonium lactate medium when an excess of T_2, T_4 or T_6 (the latter two with tryptophane) is added. An electron microscopic examination of such preparations showed that many cells had disintegrated in the presence of the virus. Naturally such preparations did not recover their original opacity after continued aeration as did those in Doermann's experiments (33). This lytic activity of T_2, but not of T_4 or T_6, could be separated from the major fraction of the virus particles by heavy ultraviolet irradiation of the virus. It would be most interesting to see whether this lytic fraction of T_2 could produce some of the effects observed by Doermann.

It is reported that the initial drop in turbidity of normal cells is not due to lysis, as shown by plaque counts (?), but is due to some change(s) in the light-scattering power of the bacteria brought about by the adsorption of the virus or its penetration into the cell. The scattering of light of the type dealt with here is, in general, due to gradients in refractive indices; differences between the refractive index of the cell or of its component parts and the refractive index of the surrounding medium are responsible for the observed scattering. A release of material from the cell, for example, could effect a reduction in the effective refractive index gradients and a reduction in light scattering.

Preliminary experiments (T. F. Anderson, unpublished) indicate that material, not sedimentable at 4000 rpm and having an ultraviolet adsorption spectrum similar to that of nucleic acid, is indeed released into the medium within five minutes after T_2 has been adsorbed on B in ammonium lactate medium. The material is released on single infection, has about five times the optical density (at 2600 Å) of the added virus and increases in amount as the multiplicity of infection is increased.

Cytological Studies. A preliminary account of microscopic studies of B:T_2 (57) indicates that soon after adsorption of T_2 the Giemsa- and Feulgen-staining "nuclear" bodies of the host disintegrate, and staining material migrates to the cell surface. The total amount of staining material then rapidly increases until the cell appears to be filled with tiny staining granules. If these granules turn out to contain high concentrations of desoxypentose nucleic acid, as might be supposed and as T_2 and in fact B:T_2 at this stage do (see *infra*), the granules might well represent nascent virus particles. It is to be hoped that a full account of these studies will soon be available, even in the absence of a logical theory of their significance.

Biochemical Syntheses. The pattern of biochemical synthesis of a host cell is altered by the adsorption of a virus particle on it, and indeed progresses through various stages as the development of the virus-host complex proceeds toward lysis and liberation of new virus. For example, Cohen finds (18) that the total protein content of the B:T_2r^+ complex increases linearly, starting at the moment of virus adsorption. In contrast, of course, a logarithmic increase is observed in uninfected host cells. However, desoxypentose nucleic acid, of which this virus contains 34 per cent (19), does not begin to accumulate until ten minutes later. Its rate of increase is then much greater than that in the uninfected host and is also linear until a point is reached at which the ratio of synthesized desoxypentose to synthesized protein is that of the virus. At this point the rates decrease and lysis begins. Ribose, an important constituent of a number of coenzymes but apparently lacking in the virus, does not accumulate during development.

From radioactive tracer experiments (18, 62*a*) it appears that some 77 per cent of the phosphorus of T_2 and T_6 virus (of which the major fraction is in desoxypentose nucleic acid) is derived from the medium, 23 per cent from the host. The results of tracer experiments using labelled hydrogen, carbon, nitrogen, oxygen, phosphorus, sulfur, *etc.*, will be awaited with interest.

A number of studies of the nutritional requirements of B:T_2 have been made using various techniques. In one of these host B are grown in nutrient broth, washed by centrifuging and suspended in synthetic (F) medium to which a single prospective growth factor has been added. When one-step growth curves are made it is found (20*b*) that average bursts on F medium alone are only 20 or less and considerably delayed, while seven amino acids added separately to F increased or accelerated the production of virus. Other naturally occurring amino acids inhibited virus synthesis in the simple medium. A "complete" medium containing naturally occurring amino acids, including those which inhibited synthesis when alone, gave one-step growth curves essentially similar to those obtained in nutrient broth.

In a second technique (35*a*) B were grown in nutrient broth, washed and infected as before, but the resulting B:T_2 allowed to develop in the complete medium from which one of the constituents was withdrawn. Nine amino acids and one purine proved to be important in determining burst size and latent period by this test. Three of these amino acids favored virus production by the previous single factor technique, while one of them inhibited virus production when present in F medium alone. Studies of this type are difficult to interpret, in particular since infected cells have been shown (57*a*) to be incapable of adapting themselves to new carbon and energy sources.

Lytic Debris. What becomes of host elements when the cell is lysed? This line of inquiry has been neglected in spite of the fact that the fates of particular enzyme systems might well depend on their participation in the development of the virus-host complex. The activities of some enzyme systems are greatly reduced on lysis. For example, the rate of oxygen consumption in an ammonium lactate medium is unaffected by infection of B with T_2 until the moment of lysis when the rate drops markedly. The survival of some enzymes depends on the nutrient or salt in the medium. Desoxyribosenuclease is found (17) in T_2 lysates in broth, but not in ammonium lactate. It is not yet clear whether this is related to the differences in burst sizes (120 vs 30) observed in the two media. It does seem clear, however, that an enzymatic analysis of lysates produced by the action of different viruses under different conditions would provide evidence for the natures of the several virus-host complexes. In addition, providing, as they do, a practically unlimited variety of tools for the breakdown of cells, the the viruses should prove invaluable in cytochemical analysis.

Action of Inhibitors. As mentioned previously, 5-methyl tryptophane (5MT) inhibits multiplication of B, and this action is reversed by addition of tryptophane (6). The compound is a satisfactory adsorption co-factor for T_4. However, B infected with either T_2 or T_4 in the presence of 5MT is unable to liberate active virus unless "rescued" by the removal of the compound or the addition of tryptophane (20). Indeed 5MT inactivates such virus-host complexes at a logarithmic rate, even though the compound has no such inactivating action on B, T_2 or T_4 treated individually. When 5MT is added to normal $B:T_2$ or $B:T_4$ and tryptophane added later the latent period is delayed for a time equal to that of the exposure to 5MT alone as though the inhibitor had stopped the metabolic machinery of phage production and lysis until its action was reversed by tryptophane (20*a*).

Certain acridines inhibit growth of a certain strain of *E. coli* and growth of a phage within the host as well (35). In these cases the inhibition of phage growth is reversed by ribose nucleic acid. It is interesting that a mutant strain of coli selected for resistance to one of the acridines proved to be resistant to lysis by the phage as well, and particularly that the phage resistance appeared to be reversed by the addition of ribose nucleic acid. It is difficult to tell at the present stage of these experiments whether the acridines blocked the adsorption process or blocked some intracellular events which follow adsorption. It seems clear that detailed studies of metabolic inhibitors and the compounds which reverse their action will contribute greatly to our understanding of virus growth, particularly if the enzymatic or other sites of their competitive action can be localized.

SUMMARY

Free virus particles can be studied directly; they have remarkably uniform morphologies within a given strain or group of serologically related strains. Alone the virus particles are inert.

They display biological activity only when adsorbed on their host cells, or, of course, when injected into animals where they elicit antibody formation. Some bacterial viruses need to take up substances (co-factors) from the medium before they are adsorbed on their hosts. Some are not adsorbed in violently agitated media. In quiet media the adsorption rate can be accounted for by diffusion of the virus to the cell surface when a large proportion of the collisions results in irreversible adsorption. Mutation of bacteria to resistance occurs in the absence of virus, and results in the loss of the adsorption mechanism for the virus in question.

Adsorption of a virus on its host results in the formation of a complex between elements of the two, an entity whose properties differ in many respects from those of either the virus particle or the host. Normal development of the complex leads to the liberation of new virus particles varying widely in number from cell to cell.

The make-up of the complex and the nature of its development have recently been studied by a number of indirect means. Some viruses can multiply on cells killed by ultraviolet or X-irradiation, but not by heat. It is inferred that elements of the host required for the formation of complexes liberating these viruses are relatively insensitive to ultraviolet or X-irradiation, but are sensitive to heat. Elements of the virus, however, are relatively sensitive to ultraviolet irradiation, and in multiple infection undamaged elements of one virus particle can substitute for the damaged elements of another to make up the complete set of virus elements which is required for the development of the complex to the point of liberation of new active virus. Related virus strains can complete each other's sets of damaged elements in this way.

Mutations of bacterial viruses have not been induced by treatment of free virus particles, but appear to occur in the development of the complex.

Mixed infection of *E. coli* by related virus strains leads to the production of hybrid types; analyses of the progeny from such crosses provide evidence for the existence of several linkage groups connecting otherwise independent elements of inheritance in these viruses.

The development of the complex has recently been followed by a number of methods. Its sensitivity to ultraviolet irradiation drops greatly five minutes after its formation and then rises again just before lysis. The sensitivity to X-rays follows a somewhat similar pattern during development of the complex but in addition reveals the existence of many intracellular centers of virus production well before finished virus particles are formed. These finished particles can be extracted by several means from the complex before it would normally lyse.

Metabolic studies show that on adsorption of T_2 virus on *E. coli* the rate of lactate oxidation remains constant, protein synthesis is linear, but desoxypentose production is delayed for five to ten minutes, at which time this substance is produced at a linear rate, greatly in excess of that observed in uninfected cells. Study of the action of metabolic inhibitors and of the compounds which block their action offers promise of elucidating many of the factors involved in the development of the virus-host complex. Enzymes and other constituents of virus-host complexes must be liberated on lysis, but their fates have so far not been adequately studied.

LITERATURE CITED

1. ANDERSON, E. H. Incidence of metabolic changes among virus-resistant mutants of a bacterial strain. Proc. Nat. Acad Sci. **30**: 397–403. 1944.
2. ————. Growth requirements of virus-resistant mutants of *Escherichia coli* strain "B". Proc. Nat. Acad. Sci. **32**: 120-128. 1946.
3. ANDERSON, T. F. Virus reactions inside of bacterial host cells. Jour. Bact. **47**: 113. 1944.
4. ————. On a bacteriolytic substance associated with a purified virus. Jour. Cell. & Comp. Physiol. **25**: 1–15. 1945.
5. ————. The role of tryptophane in the adsorption of two bacterial viruses on their host *E. coli.* Jour. Cell. & Comp. Physiol. **25**: 17–26. 1945.
6. ————. The activity of a bacteriostatic substance in the reaction between bacterial virus and host. Science **101**: 565–566.
7. ————. Morphological and chemical relations in viruses and bacteriophages. Cold Spring Harbor Symp. Quant. Biol. **11**: 1–13. 1946.

7*a*. ————. The activation of the bacterial virus T4 by *L-tryptophan.* Jour. Bact. **55**: 637–649. 1948.

7*b*. ————. The inheritance of requirements for adsorption cofactors in the bacterial virus T4. Jour. Bact. **55**: 651–658. 1948.

7*c*. ————. The influence of temperature and nutrients on plaque formation by bacteriophages active on *Escherichia coli* strain B. Jour. Bact. **55**: 659–665. 1948.

7*d*. ———— *et al.* The relative sensitivity of bacterial viruses to sonic vibration. Science **108**: 18–19. 1948.

7*e*. ———— and SHETZLEY, C. M. [Unpublished results].

8. ———— *et al.* Types of morphology found in bacterial viruses. 2nd Ann. Meet. Electron Micr. Soc. Am., Chicago, Nov. 17, 1944.
9. ASHENBERG, N. J. *et al.* The influence of bacterial and non-bacterial polysaccharides upon bacteriophagy. Jour. Bact. **39**: 71–72. 1940.
10. BAWDEN, F. C. Plant viruses and virus diseases. 2nd ed. 1943.

10*a*. BEARD, J. W. The chemical, physical and morphological properties of animal viruses. Physiol. Rev. **28**: 349–367. 1948.

11. BERNAL, J. D. and FANKUCHEN, I. X-ray and crystallographic studies of plant virus preparations. Jour. Gen. Physiol. **25**: 111-165. 1941.
12. BURNET, F. M. The phage-inactivating agent of bacterial extracts. Jour. Path. & Bact. **38**: 285–299. 1934.
13. ————. The bacteriophages. Biol. Rev. **9**: 332–350. 1934.
14. ————. Virus as organism. Evolutionary and ecological aspects of some human virus diseases. 1945.

15. ———— *et al.* The immunological reactions of the filtrable viruses. Australian Jour. Exp. Biol. & Med. Sci. **15:** 227–368. 1937.

15*a*. ———— *et al.* Mucin as substrate of enzyme action by viruses of the memps influenza group. Nature **160:** 404–405. 1947.

16. ———— *et al.* Modification of human red cells by virus action. I. The receptor gradient for virus action in human red cells. Brit. Jour. Exp. Path. **27:** 228–236. 1946.

17. Cohen, S. S. Streptomycin and desoxyribonuclease in the study of variations in the properties of a bacterial virus. Jour. Biol. Chem. **166:** 393–394. 1946.

18. ————. The synthesis of bacterial viruses in infected cells. Cold Spring Harbor Symp. Quant. Biol. **12:** 35–49. 1947.

18*a*. ————. The synthesis of bacterial viruses. II. The origin of the phosphorus found in the desoxyribonucleic acids of the T2 and T4 bacteriophages. Jour. Biol. Chem. **174:** 295–303. 1948.

19. ———— and Anderson, T. F. Chemical studies on host-virus interactions. I. The effect of bacteriophage adsorption on the multiplication of its host, *Escherichia coli B.* With an appendix giving some data on the composition of the bacteriophage, T_2. Jour. Exp. Med. **84:** 511–523. 1946.

20. ———— and ————. Chemical studies on host-virus interactions. II. The chemical simulation of the interference phenomenon by 5-methyl tryptophane. Jour. Exp. Med. **84:** 525–533. 1946.

20*a*. ———— and Fowler, C. B. Chemical studies on host-virus interaction. III. Tryptophan requirements in the stages of virus multiplication in the *Escherichia coli*-T2 bacteriophage system. Jour. Exp. Med. **85:** 771–784. 1947.

20*b*. ———— and ————. Chemical studies on host-virus interaction. V. Some additional methods of determining nutritional requirements for virus multiplication. Jour. Exp. Med. **87:** 275–282. 1948.

21. Darlington, C. D. Heredity, development, and infection. Nature **154:** 164–169. 1944.

22. Delbrück, M. Adsorption of bacteriophages under various physiological conditions of the host. Jour. Gen. Physiol. **23:** 631–642. 1940.

23. ————. Bacterial viruses (bacteriophages). Advances in Enzymology **2:** 1–32. 1942.

24. ————. Growth of bacteriophages. Ann. Rep. Biol. Lab., Long Island Biol. Ass'n. **55:** 21–22. 1944.

25. ————. The burst size distribution in the growth of bacterial viruses (bacteriophages). Jour. Bact. **50:** 131–135. 1945.

26. ————. Effect of specific antisera on the growth of bacterial viruses (bacteriophages). Jour. Bact. **50:** 137–150. 1945.

27. ————. Interference between bacterial viruses. III. The mutual exclusion effect and the depressor effect. Jour. Bact. **50:** 151–170. 1945.

28. ————. Bacterial viruses or bacteriophages. Biol. Rev. **21:** 30–40. 1946.

29. ————. Experiments with bacterial viruses (bacteriophages). The Harvey Lecture Series **41:** 161–187. 1946.

30. ———— and Bailey, W. T., Jr. Induced mutations in bacterial viruses. Cold Spring Harbor Symp. Quant. Biol. **11:** 33–37. 1946.

31. ———— and Luria, S. E. Interference between bacterial viruses. I. Interference between two bacterial viruses acting upon the same host, and the mechanism of the virus growth. Arch. Biochem. **1:** 111–141. 1942.

32. Demerec, M. and Fano, U. Bacteriophage-resistant mutants in *Escherichia coli.* Genetics **30:** 119–136. 1945.

33. Doermann, A. H. [Discussion of paper by A. D. Hershey]. Cold Spring Harbor Symp. Quant. Biol. **11:** 76–77. 1946.

33a. ————. [Personal communication].

33b. ———— and Anderson, T. F. [Unpublished results].

34. Ellis, E. L. and Delbrück, M. The growth of bacteriophage. Jour. Gen. Physiol. **22**: 365–384. 1939.

35. Fitzgerald, R. F. and Lee, M. E. Studies on bacterial viruses. II. Observations on the mode of action of acridines in inhibiting lysis of virus-infected bacteria. Jour. Immunol. **52**: 127–135. 1946.

35a. Fowler, C. B. and Cohen, S. S. Chemical studies on host-virus interaction. IV. A method of determining nutritional requirements for bacterial virus multiplication. Jour. Exp. Med. **87**: 259–274. 1948.

36. Freeman, M. The phage inactivating agent in extracts of *Staphylococcus aureus*. Australian Jour. Exp. Biol. & Med. Sci. **15**: 221–225. 1937.

37. Gough, G. A. C. and Burnet, F. M. The chemical nature of the phage-inactivating agent in bacterial extracts. Jour. Path. & Bact. **38**: 301–311. 1934.

38. d'Herelle, F. Sur un microbe invisible antagoniste des bacilles dysentériques. Comp. Rend. Acad. Sci., Paris **165**: 373–375. 1917.

39. ————. The bacteriophage and its behavior. 1926.

40. Hershey, A. D. Mutation of bacteriophage with respect to type of plaque. Genetics **31**: 620–640. 1946.

41. ————. Spontaneous mutations in bacterial viruses. Cold Spring Harbor Symp. Quant. Biol. **11**: 67–76. 1946.

41a. ———— and Rotman, R. Linkage among genes controlling inhibition of lysis in a bacterial virus.

42. Howe, H. A. and Bodian, D. Neural mechanisms in poliomyelitis. 1942.

43. Kalmanson, G. M. and Bronfenbrenner, J. Restoration of activity of neutralized biological agents by removal of the antibody with papain. Jour. Immunol. **47**: 387–407. 1943.

44. Knight, C. A. Precipitin reactions of highly purified influenza viruses and related materials. Jour. Exp. Med. **83**: 281–294. 1946.

45. ————. Nucleoproteins and virus activity. Cold Spring Harbor Symp. Quant. Biol. **12**: 115–121. 1947.

45a. Latarjet, R. Intracellular growth of bacteriophage studied by Roentgen irradiation. Jour. Gen. Physiol. **31**: 529–546. 1948.

46. ———— and Wahl, R. Precisions sur l'inactivation des bacteriophages par les rayon ultraviolets. Ann. Inst. Pasteur **71**: 336–339. 1945.

47. Luria, S. E. A growth-delaying effect of ultraviolet radiation on bacterial viruses. Proc. Nat. Acad. Sci. **30**: 393–397. 1944.

48. ————. Mutations of bacterial viruses affecting their host range. Genetics **30**: 84–99. 1945.

49. ————. Genetics of bacterium-bacterial virus relationship. Ann. Missouri Bot. Garden **32**: 235–242. 1945.

50. ————. Spontaneous bacterial mutations to resistance to anti-bacterial agents. Cold Spring Harbor Symp. Quant. Biol. **11**: 130–137. 1946.

51. ————. Recent advances in bacterial genetics. Bact. Rev. **11**: 1–40. 1947.

52. ————. Reactivation of ultraviolet inactivated bacteriophage particles inside double-infected host cells. Soc. Am. Bact., Abs. Proc., 47th Gen. Meet., May 13–16. 1947.

53. ———— and Anderson, T. F. The identification and characterization of bacteriophages with the electron microscope. Proc. Nat. Acad. Sci. **28**: 127–130. 1942.

54. ———— and Delbrück, M. Interference between inactivated bacterial virus and active virus of the same strain and of a different strain. Arch. Biochem. **1**: 207–218. 1942.

55. ———— and ————. Mutation of bacteria from virus sensitivity to virus resistance. Genetics **28:** 491–511. 1943.
56. ———— *et al.* Electron microscope studies of bacterial viruses. Jour. Bact. **46:** 57–77. 1943.
57. ———— and LATARJET, R. Ultraviolet irradiation of bacteriophage during intracellular growth. Jour. Bact. **53:** 149–163. 1947.
57*a*. MONOD, J. and WOLLMAN, E. L'inhibition de la croissance et de l'adaptation enzymatique chez les bactéries infectées par le bactériophage. Ann. Inst. Pasteur **73:** 937–957. 1947.
58. MULLER, H. J. Resumé and perspectives of the symposium on genes and chromosomes. Cold Spring Harbor Symp. Quant. Biol. **9:** 290–308. 1941.
59. ————. The gene. Proc. Royal Soc. London (B) **134:** 1–37. 1947.
60. PAULING, L. and PRESSMAN, D. The serological properties of simple substances. IX. Hapten inhibition of precipitation of antisera homologous to the *o*-, *m*-, or *p*-Azophenylarsonic acid groups. Jour. Am. Chem. Soc. **67:** 1003–1012. 1945.
61. PIRIE, A. The effect of lysozyme on the union between a phage and the susceptible *Bacillus megatherium*. Brit. Jour. Exp. Path. **21:** 125–132. 1940.
62. POISON, S. D. Recherches sur la probabilitié des jugements en matière criminelle et en matière civile, precedées des regles génerales du calcul des probabilités. 1937.
62*a*. PUTNAM, F. W. and KOZLOFF, L. M. On the origin of virus phosphorus. Science **108:** 386–387. 1948.
63. ROTHEN, A. Immunological reactions between films of antigen and antibody molecules. Jour. Biol. Chem. **168:** 75–97. 1947.
64. ROUYER, M. and LATERJET, R. Augmentation du nombre de bacteriophages en presence des bactéries sterilisées par irradiation. Ann. Inst. Pasteur **72:** 89–94. 1946.
65. SCHLESINGER, M. Über die Bindung des Bakteriophagen an homologe Bakterien. I. Die Unterscheidung von Grupper verschiedener Bindungsaffinität innerhalb der Bakteriophagen desselben Lysate. Die Frage der Reversibilitat oder Irreversibilitat der Bindung. Zeits. Hygiene **114:** 130–148. 1932.
66. ————. Über die Bindung des Bakteriophagen an homologe Bakterien. II. Quantitative Untersuchungen über die Bindungsgeschwindigkeit und die Sättingung. Berechnung der Teilchengrosse des Bakteriophagen aus deren Ergebnissen. Zeits. Hygiene **114:** 149–160. 1932.
67. v. SMOLUCHOWSKI, M. Versuch einer mathematischen Theorie der Koagulationskinetik kolloider Lösungen. Zeits. Physik. Chem. **92:** 129–169. 1917.
68. STANLEY, W. M. and ANDERSON, T. F. A study of purified viruses with the electron microscope. Jour. Biol. Chem. **139:** 325–345. 1941.
69. ———— and ————. Electron micrographs of protein molecules. Jour. Biol. Chem. **146:** 25–33. 1942.
70. STONE, J. D. Enzyme modification of the reaction between influenza virus and susceptible tissue cells. Nature **159:** 780–781. 1947.
71. TWORT, F. W. An investigation of the nature of ultra-microscopic viruses. Lancet **2:** 1241–1243. 1915.
72. WILLIAMS, R. C. and WYCKOFF, R. W. G. Electron shadow micrography of virus particles. Proc. Soc. Exp. Biol. & Med. **58:** 265–270. 1945.
73. WITKIN, E. M. Inherited differences in sensitivity to radiation in *Escherichia coli*. Proc. Nat. Acad.. Sci. **32:** 59–68. 1946.
74. WYCKOFF, R. W. G. Some biophysical problems of viruses. Science **101:** 129–136. 1945.

THE BOTANICAL REVIEW

VOL. XV OCTOBER, 1949 No. 8

THE HETEROCHROMATIN PROBLEM IN CYTOGENETICS AS RELATED TO OTHER BRANCHES OF INVESTIGATION[1]

LEON VANDERLYN
Biological Abstracts

INTRODUCTION 508
THE PERIPHERAL LOCATION OF CHROMATIN AND HETEROCHROMATIN 508
THE NATURE AND FUNCTIONS OF HETEROCHROMATIN 511
  Definitions 511
  Regions and Structures Described as Heterochromatic 513
  Characteristics Attributed to Heterochromatin 514
    Heteropycnocity 514
    Allocycly 514
    Sensitivity to External Conditions 514
    Genetic Inertness 514
    Changes 514
    Absence or Presence in Chiasmata Regions 515
    Concentration in the Centromere Area 515
    Tendency to Cause Euchromatic Changes 516
    Tendency to Divide Slowly 516
    Tendency to Flow or Clump Together 516
    Spiralization 517
    Alveolar or Linear Structure 517
    Existence in Two Types 519
  Functions Attributed to Heterochromatin 520
    Genetic Inertness 520
    Regions of Synthetic and Metabolic Phenomena 523
    Transformation of Euchromatic Regions 526
    Other Functions 528
CENTROMERES AND TELOMERES 531
PHYSICAL AND CHEMICAL PROPERTIES OF NUCLEIC ACID 534
HETEROCHROMATIN CONSIDERED IN THE FRAMEWORK OF CELL POLARITY 543
A NEW DEFINITION OF HETEROCHROMATIN 549
  Heterochromatin as Liaison Chromatin 552
  The Nature of the Definition as an Hypothesis 556
  Nucleic Acid as Concerned with Energy-Metabolism 559
  The Nature of Enzymes as Related to the Problem of Heterochromatin—The Polygene Concept 566
    Heterochromatin and Neoplasia 570
    Non-homologous Associations 571
SUMMARY—The Cell as an Energy Transformer 572
LITERATURE CITED 576

[1] This article is a sequel to that by the same author in THE BOTANICAL REVIEW 14: 270–318. 1948, and was submitted for publication in a preliminary form before Mr. Vanderlyn died. The arduous task of completing various details in it, particularly that of verifying the bibliography and checking the references in the text to it, was undertaken by Dr. Jean McCreight, Mr. Vanderlyn's associate, and to her the editor and readers of the REVIEW are much indebted for making publication possible.

INTRODUCTION

It frequently happens that the most controversial frontiers of scientific research act as a focal point, integrating the findings in many fields of investigation. This is strikingly true of the heterochromatin problem which, while fundamentally cyto-genetic, cuts across almost all aspects of cell function and structure. The nature of the cell is an unknown substrate into which the separate disciplines of biological research have been tunnelling for over one hundred years. When a tunnel is engineered through a mountain or in a river bed, the workers on both sides are aware that they are to meet quite precisely and usually at a scheduled time. In biological research we do not know, in starting out, exactly in which direction we are digging with relation to the other branches of investigation, and the meeting points are often a surprise to everyone, as has been the case when members of the vitamin-B complex were shown to be connected with the respiratory enzyme system. Within the last decade there has been a spectacular union of many separate tunnels, and this union, it is the purpose of this review to point out, if properly appreciated, and what is even more important, if properly taught, may provide us with an enriched and simplified view of the nature of the cell.

The first point of union was that between cytology and genetics. Early in the twentieth century it became recognized that the behavior of the chromosomes in the complex cycle of meiosis, syngamy and somatic mitosis parallels not only the essential facts of the hereditary continuity and equipartition of parental traits but also the Mendelian laws of segregation and independent assortment. With critical localization of specific factors on definite chromosomes, the science of cyto-genetics came into existence. There have been numerous other points of conjunction, and, as I shall show, they are directly or indirectly related to the heterochromatin problem.

THE PERIPHERAL LOCATION OF CHROMATIN AND HETEROCHROMATIN

In a preceding paper in this journal (145*a*) I pointed out that much of the speculation concerning the nature and function of heterochromatin has taken place at the purely genetic and biochemical level without reference to its topography and distribution. As a preliminary to a reconsideration of this topic in terms of its allocation in the cell, I stressed the following points: (*a*) that centromeres and often telomeres are associated with the nuclear membrane, and the nucleogenic regions with the nucleolar surface; (*b*) that these regions are frequently heterochromatic; (*c*) that, conversely, the bulk of heterochromatin occurs in these regions; and (*d*) that to judge from their topography chromosomes appear to be tracks or lines of transfer of energy and/or substance between the nucleolus and cytoplasm.

From whatever point we look at it, the mitotic cycle is one of expansion and contraction in the degree of surface presented by the nuclear chromatin. Whether the metabolic stage is alveolar, reticular or a mass of minor chromonemal coils, it is a maximum in surface area and in labyrinthine intricacy of structure. Whether or not we view the solid metaphase chromosome as a condensation from a previous diffuse state, or as a compact result of maximum spiralization, metaphase, with its accompanying dissolution of nuclear and nucleolar membranes, is a time of minimum surface area and great simplicity in external structure and configuration. For the reticular type of nucleus at least, the nucleic acid cycle consists of alternate expansion (by dispersion or by uncoiling) and contraction (by condensation or by tighter coiling) in surface area; regions at or near the nuclear and nucleolar membranes (heterochromatin) are allocyclic with respect to regions farther from these membranes (euchromatin), *i.e.*, they show differences in their nucleic acid cycle, which are in all probability a consequence of their more immediate relationship with the cytoplasmic and nucleolar environments.

In *Allium*, I pointed out, this tendency for the more chromatic regions to be located predominantly at the periphery is also true of chromosome structure. Where spirals are seen, the gyres are for the most part applied against the outer boundary of the matrix. Chromatic granules are also conspicuously larger and more frequent at this boundary. Again, as the chromosomes attain their maximum metaphase chromaticity, they tend to become more and more aligned against the nuclear membrane. This property of peripheral, or membrane surface, location of heterochromatin is apparently shared by chromatin itself in the condensed stage.

A survey of the literature reveals some interesting data on this point. As noted in the previous paper, the tendency of isolated chromosomes to form karyomeres is quite common. Those of *Fundulus* show a predominantly peripheral distribution of chromatin (130). In the vesicles of *Vivipara* the chromatin is heavily concentrated to one edge (119). It is with great frequency depicted as circumferentially arranged in telophase reorganization [(145) p. 11, Fig. 2 for *Ascaris;* (87) Plate I, Fig. 4 for *Nereis*]. In the characteristic protruding lobes of the *Ascaris* nucleus, the chromosomes coil along the margins [(145), Plate XII, Figs. 34, 36, 37*a–c*]. In the atypical spermatogenesis of the Iceryidae recently described by Hughes-Schrader (73), the chromosomes of the haploid set become closely appressed against the nuclear membrane as they undergo telophase despiralization (see especially her Fig. 4). A similar appressment against the nuclear membrane occurs in vesiculation following fertilization in these forms. These are citations from normal conditions, and the list could be added to extensively. Such peripheral distribution is also known to occur under abnormal conditions. A frequent accumulation of chromatin at the nuclear membrane occurs in cells of the frog embryo exposed to hexenolactone (18).

The honeycomb or vascuolar structure described for the giant salivary gland chromosomes (47, 79, 81) may be part of this picture, with the chromatin arranged at the periphery of achromatic vacuoles; the same may be true of the heterochromomeres described for *Chironomus* (2, 4) and *Drosophila* (5), consisting of an achromatic core and a chromatic hull. It is also interesting to note a lecture report by Dr. Seymour Cohen (29) to the effect that the nucleic acid of the phage viruses is also located at the surfaces of these structures.

Similar behavior on the part of whole nuclei may be related to this phenomenon. Movement of the nuclei to the surface of the egg in the formation of polar bodies; the drift of blastomeres to the surface of the insect egg; migration of the dividing cells of the plant embryo to its outer margins and of nuclei in tissues adjacent to the vicinity of a wound; the location of cell nuclei in the outer region of secretory glands; frequent peripheral distribution of the nuclei in cysts of oogonia and spermatogonia; the radiate arrangement of spermatids so that their nuclei lie adjacent to the cyst wall (Orthoptera, Hemiptera, *etc.*); the circumferential pattern assumed by the trophozoites of *Plasmodium falciparum,* with the chromatin granule at the surface of the blood cell—all these may have as common denominator some as yet unexplained function of chromatin or nucleic acid. Again, I find it of interest that the Donovan bodies, of granuloma inguinale, which are not yet suitably classified, often exhibit a similar cortical distribution as they multiply and mature in the endothelial monocytes [(58), Fig. 12, 4].

We have, therefore, a broad and undoubtedly significant character of this essential substance. Chromatin at all of its levels of integration seems to be associated with the outer surface of the structures of which it is part.

THE NATURE AND FUNCTIONS OF HETEROCHROMATIN

By far the greater part of thinking and speculation on heterochromatin has been at the genetic and biochemical level, mostly in genetic terms and in connection with genetic phenomena. There is, moreover, a great deal of confusion over just what heterochromatin is, and which regions or structures are heterochromatic. The following is a synopsis of the problem, summarizing (I) definitions of heterochromatin, (II) regions and structures described as heterochromatic, (III) characteristics attributed to heterochromatin, and (IV) functions attributed to heterochromatin. All these points will be reconsidered in the light of the location of heterochromatin in the cell, and of its behavior in the mitotic cycle. An attempt will be made to show which definitions and which hypotheses are supported by this type of evidence and which it places in jeopardy.

I. Definitions

Before even considering its subdivision into eu- and heterochromatin, we should be certain of the exact meaning of the term "chromatin" itself. Heitz has reviewed this problem (64). He cites Boveri's definition of chromatin as "the substance in the nucleus which in mitosis transforms into chromosomes, whether strongly stainable, weakly stainable, or not at all stainable". He also quotes Belar's remark that "chromatin is a matter of neither morphology nor staining, but a morphogenetic entity". It is one of those unfortunate malapropisms in nomenclature that a substance which can be either chromatic or achromatic should be called "chromatin" merely because it was originally identified by its affinity for basic dyes at certain stages of the mitotic cycle. At present, using the Feulgen technique as standard, chromaticity is identified with the nucleic acid content of chromatin; conversely, when chromatin is achromatic, its nucleic acid content is presumably low, or obscured by dispersion.

The terms "euchromatin" and "heterochromatin" are naturally confined to chromonucleic acid, since they are intranuclear phenomena. Heitz first introduced this terminology (63). By his original definition, euchromatin is that chromosome substance which undergoes the customary telophase dispersion or dissolution. Heterochromatin, on the other hand, is that chromosome substance retaining its maximum metaphase condensation, or chromaticity. No difference between the two types is detectable at metaphase either in stained preparations or in the living condition; nor does the Feulgen reaction discriminate between the two. The distinctions are apparent in prophase, resting stage and telophase. In its original sense, then, "heterochromaticity" is synonymous with the older term "heteropycnocity" first employed by Gutherz (60) to describe heterochromosomes.

Schultz (134) more or less follows this in defining heterochromatin as "The regions of the chromosomes which stain most deeply at interphase. . . ." Pontecorvo's definition (120) is ". . . chromosomes or chromosome segments which respond to staining during mitosis in a different way *from the rest of the set taken as a standard*". (The italics are mine.)

Darlington and La Cour (37) define heterochromatin as "those parts of chromosomes which have the inherent property of failing to maintain the maximum nucleic acid cycle at mitosis. . .". They believe the visible difference between eu- and hetero-chromatin to be a difference in the timing and/or amplitude of the cyclic staining reaction of prophase synthesis and telophase dissolution of chromonucleic acid. This allocycly is of two types, overcondensation and undercondensation.

White (149) uses the terms "positive" and "negative heterochromatin" to describe these two states. He associates the former with the tightening of the chromonemal spiral, and the latter with its loosening up, although that "thickening and spiralization are to some extent independent and not merely aspects of the same phenomenon".

Again, somewhere down the line in the history recounted immediately above and under *IV* below, the idea became established that heterochromaticity is distinguishable from heteropycnocity by the quality of genetic inertness. There was never any formal statement of this dichotomy. What happened was that a pronounced tendency to think in genetic terms effected a translocation of meaning. Genetic inertness became synonymous with heterochromaticity, though many of the structures from which the original definition of the term came were never shown to be inert. And conversely there are examples where heterochromatic regions are genetically active (see *IV* A below).

II. Regions and Structures Described as Heterochromatic

A. Parts of chromosomes

1. Chromocenters
    - (*a*) individual chromocenters (*e.g.*, distal chromocenters of *Allium*)
    - (*b*) compound chromocenters (*Drosophila* salivary gland nucleus)
2. euchromocenters (prochromosomes) (euchromocentric nuclei of plants)
3. polar granules (*e.g., Phrynotettix*) (146)
4. polar caps (*e.g., Batracoseps* and *Stauroderus*) (76, 33)
5. conditions intermediate between 1 and 4 (*e.g.*, proximal chromocenters of *Allium*)
6. trabants
7. nucleolus-organizing regions (*e.g., Zea*) (97)
8. detached nucleolus-organizers of the *Bufo* oocyte (114)
9. bulbs and puffs and regions producing the Neben-nucleoli of *Chironomus* and *Sciara* (4, 6, 122)
10. Balbiani's ring (*Chironomus*) (4, 6, 122, 2)
11. intercalary heterochromatin, as in salivary gland chromosomes of various Diptera (*e.g., Drosophila pallidipennis*) (44)
12. chromomeres

B. Whole chromosomes

1. sex chromosomes
2. megameric chromosomes (Orthoptera) (146, 21)

3. supernumerary chromosomes (*e.g.*, B-chromosomes of *Zea*) (90, 82)
4. limited chromosomes (*Sciara*) (99)
5. compound chromosomes (*e.g.*, *Gossyparia*) (131)

III. Characteristics Attributed to Heterochromatin

A. Heteropycnocity

B. Allocycly (37)

C. Sensitivity to External Conditions. This has been discussed extensively in the recent paper; such sensitivity may express itself as:

1. increase in size or prominence of heterochromatic areas;
2. disturbance in allocycly; differences in allocycly according to sex or tissue belong under this head (120);
3. effects attributable to heterochromatin, as increase in nuclear size, *etc.*, which are treated under *IV* below.

D. Genetic Inertness. The criteria are:

1. deficiencies and duplications of inert chromosomes; segments without marked phenotypic effect (120, 38);
2. absence or rarity in these regions of spontaneous or induced mutations (38), interpreted as an absence or rarity of genes "detectable by sharply alternative effects of different allelomorphs"; this can more logically and sequentially be treated under the functions of heterochromatin. The subject will therefore be more extensively considered under *IV* (120) below.

E. Changes. Although deficient in spontaneous and induced mutations, on the basis of cytologic and genetic evidence, heterochromatin has been thought to undergo both spontaneous and induced structural change more readily than other parts (40, 39, 78, 126, 7, 108, 109, 125). Mather (93) finds that while crossing over in both eu- and hetero-chromatin is dependent on the distance from the centromere, "the high sensitivity of crossing over to temperature is a property of heterochromatin". It has also been suggested that there may be some correlation between this frangibility and the fragility of heterochromatin under cytological treatment. E may be considered a corollary of C above.

F. Absence or Presence in Chiasmata Regions. Darlington's suggestion that heterochromatin tends to appear in regions where chiasmata are rare—since where chiasmata are absent, recombination is likewise absent and genes would therefore mutate to inertness in these regions—has been reviewed by Callan (20) who finds the situation much more complex, some cases showing an absence of chiasmata in heterochromatic regions, while in others chiasmata form in the heterochromatin. Callan states that Darlington has made the additional proviso that in the latter cases the heterochromatin in the chiasmata-forming regions is "adapted to regulate the crossover frequency in active regions nearby". My own belief is that in the matter of chiasmata we are on very uncertain ground. The problem is paralleled by that of formation of chiasmata in centromere and telomere regions. In some cases chiasmata are formed in these regions; in others they are not. It is very difficult to decide whether we are dealing with the properties of a locus or a condition of the chromatin. Again, actual chiasmata are very difficult to separate from the unwinding of relational coils, which takes place through meiosis. I therefore consider this property as among the least clearly established of the many which have been described for heterochromatin.

G. Concentration in the Centromere Area. A tendency has been noted for heterochromatin to be concentrated in the centromere region (38, 20). Dobzhansky (44) cites this as the norm for the genus *Drosophila,* with certain variations and exceptions. Levan (85) finds this true of the heterochromatin of *Allium,* which confirms the deduction that the centromeres are located at the nuclear membrane in the reticulate, as they are in the euchromocentric, type of plant nucleus, since these proximal heterochromatic blocks maintain visible association with the nuclear membrane throughout interphase and early prophase. Even the tiny chromosomes of the fungi may exhibit the same arrangement. In *Neurospora* the two main heterochromatic segments are located adjacent to the centromere (98).

This whole tendency has been stated in another way in reports "that sections adjacent to the centromere in mitotic prophase figures are heteropycnotic" (71, 34, 64, 65, 149). On the other hand, the concentration has been reported as at the distal end in *Trillium* (37). The overall picture, as stated by Schultz (134), is that "chromocenters . . . are most evident at the spindle fiber attachment and at the ends of the chromosomes".

H. Tendency to Cause Euchromatic Changes. Heterochromatin has been described as causing euchromatic changes, with consequent disturbance of genetic function (111, 22, 126). Others have failed to repeat these results, finding instead a random variation which is unaffected except in one case, a known band translocated to the vicinity of the chromocenter. Dobzhansky (44), however, reports such influences as exerted even under normal conditions. Euchromatic portions of the X in *Drosophila pallidipennis,* which are immediately distad of the large proximal heterochromatin block imbedded in the chromocenter, show "considerable fluctuation in the boundary between euchromatic and heterochromatic parts", and "the rate of nucleination of the euchromatic sections seems to depend upon proximity to the heterochromatic ones". Prokofyeva-Belgovskaya (125) states for *D. melanogaster* that "The general morphology of the inert region of the X can in some cases be profoundly modified due to the proximity of the chromocenter". Caspersson and Schultz (22) show experimentally that the increase in nucleic acid content in euchromatic regions transplanted next to heterochromatic regions is proportional to the intervening distance. That is, an increase occurs which is greatest close to the heterochromatic regions and less further away. See, however, Schultz's later views under *IV* E.

I. Tendency to Divide Slowly. Heterochromatic regions have been described as dividing more slowly than euchromatic regions (135, 10, 53, 151).

J. Tendency to Flow or Clump Together. A tendency has many times been noted for heterochromatic regions to flow or clump together, as in Dipteran chromocenters and the polar cap of *Stauroderus* (33); or for non-homologous chromosomes to associate at their heterochromatic regions. This has been attributed to a certain property of "stickiness" and also to the presence of "similar" genes in these regions. On the other hand, and of especial interest in connection with G above, Ribbands (129) has amassed considerable data to show that in at least one form, the association is really by centromere and telomere ends located on the nuclear membrane rather than due to heterochromatin.

Somewhat conversely, a lack of complete contact has been noted in totally heterochromatic chromosomes, which has been designated "conjugation at a distance"; again, these chromosomes differ in the duration of synapsis. The literature on these topics is reviewed by Resende (128). His own conclusion is that these phenomena are merely aspects of the asynchrony of allocyclic behavior, and that in the last analysis, euchromatic and heterochromatic synapses are identical.

K. Spiralization. The idea of heterochromaticity as correlated with degree of spiralization (149) has not received much support. Darlington and La Çour (37) find heterochromatin segments the same length when under as when normally charged, and conclude that "nucleic acid seems to have no necessary relation to spiralization". Muller and Prokofyeva (104) suggest that heterochromatic regions are devoid of spiralization, which would tie in with the supposed dependence of "stickiness" upon non-polymerization. Callan (20) reviews the question and, on the basis of further evidence, concludes that spiralization may or may not occur in heterochromatin, according to the species and the stage of the nucleic acid cycle, and that "if it is utterly deficient in nucleic acid throughout the cell cycle it cannot spiralize". The spiralization cycle is connected with L below.

L. Alveolar or Linear Structure. If one analyzes the various descriptions of the finer structure of the heterochromatin of the salivary gland chromosomes, it will be clear that it manifests the same peculiarity as euchromatin in showing under different fixations, and possibly with differing interpretations, sometimes an alveolar appearance with darker material surrounding achromatic droplets [*e.g.*, Fig. 2*a* (64)]; sometimes a linear structure with chromomere-like bodies and intercalating strands [*e.g.*, Fig. 2 (125)]; and sometimes a stage between these with alveoles and intercalating threads [*e.g.*, Fig. 5 (5)]. The pictures of Heitz (64), Bauer (4, 6) and Poulson and Metz (122) all confirm the latter author's opinion that "since it is known that both chromatic and heterochromatic regions behave the same with Feulgen reaction, the difference lies in the distribution of the chromatic material with respect to the achromatic". These all seem to illustrate an alveolar interpretation for the heterochromomeres, as these were first named (6). Prokoyeva-Belgovskaya's picture (125) of chromonema with granules, the latter in regular alignment and forming, as would be expected of homologous chromomeres, continuous rows across the synapsing X and Y, seems to support her supposition that "the chromonema of the Y chromosome and that of the X chromosome have essentially the same structure as the chromonema of euchromatic parts of the chromosome". Moreover, she finds the distance between the chromomeres of the Y-chromosome to be the same as that between the discs of the euchromatic parts of the chromosomes—*i.e.*, that the number of chromomeres per unit length is the same in the heterochromatic Y as in the euchromatic regions of the autosomes. This will be seen to reflect the view (17) (81) that the salivary gland chromosomes are polytene structures, the darker bands of which are formed by the parallel alignment of Feulgen-positive chromomeres. The interpretation of both eu-and heterochromatin in *Simulium* (117) is that both consist of chromomeres varying from small solidly chromatic granules to alveoles with a chromatic hull and achromatic core, strung out like beads on chromatic strands, with the heterochromatic regions more spread out, and with their chromomeres more irregular in size and spacing than the euchromatic.

This picture, together with those of Metz and Poulson, seems to open to serious question the distinction (6) between euchromomeres as compact granules and heterochromeres as alveoles. It also jeopardizes, at the visible level, Pontecorvo's theory (120) that the lesser differentiation of heterochromatin is due to its being made up of "repeated replicas of a single type chromomere", and his accompanying theory of allocycly as owing to the nucleic acid cycle in euchromatin "as an expression of an average of differing chromomeres", while that of heterochromatin is "pure", and hence out of step. If we consider his "chromomeres" as the invisible genes, however, the theory might conceivably be maintained.

The picture of the finer structure of heterochromatin is thus one of variability, relativity and complexity, and not a little confusion. As noted, in these respects it is not much different from the euchromatic areas of the salivary gland chromosomes, about whose finer structure there are likewise many different interpretations. On the other hand, on genetic grounds, heterochromatin has been assumed to be less differentiated than euchromatin (120). Caspersson and his co-workers, on the basis of ultra-violet absorption data, consider heterochromatin to be made up of identical or similar genes, and that the greater uniformity is due to the simpler histone composition of the protein moiety as opposed to the globulin nature of euchromatic proteins (135, 136).

M. Existence in Two Types. Heterochromatin has been described as consisting of two types. The distinction between a permanent and a temporary heterochromatin has already been noted. Resende (128) discusses this dichotomy with examples from his own experience which lead him to believe that the temporary chromocenters are distinguishable from the permanent solely by their behavior.

Heitz (66) describes the heterochromatin of *Drosophila virilis* as occurring in the form of a more compact alpha type heterochromatin and of a more diffuse beta heterochromatin. The alpha type is apparently identical with the compact chromocenter appearing in the somatic cells, while the looser beta heterochromatin is invisible in somatic nuclei and may be formed terminally during the growth of the salivary gland chromosomes. The alpha type apparently does not undergo growth in the salivary gland nuclei and forms only a small compact and proximal part of the chromocenter. The beta type constitutes the bulk of the chromocenter and the bases of the chromosomes. Heitz believes that alpha heterochromatin is genetically inert. Pavan (118) currently reviews this problem in reporting two distinguishably different types of heterochromatin in *Drosophila nebulosa.* One type shows "relatively little in the salivary gland chromosomes in proportion to the euchromatin in the mitotic chromosomes", while the other becomes reduced from a considerable area in the mitotic chromosomes to merely a few heterochromomeres in the salivary gland nuclei. He states that no homology can be drawn between these and the alpha and beta chromatin of Heitz because the latter types cannot be localized. A similar reduction in the extent of a definite area in the chromosomes of the salivary nuclei over that occupied by the same region in somatic nuclei has reported by other workers (71, 106).

This will be discussed more fully under the functions of heterochromatin. In studies of the abnormal lampbrush structure of *Drosophila* induced in salivary gland chromosomes by treatment with urea and NaOH, differences have been detected between the terminal heterochromatin and that of intercalary regions. Other cases where two types of heterochromatin occur are discussed by Resende (153).

It has been suggested that heterochromatin is merely euchromatin protected by kalymma which inhibits genetic action and protects the chromonemata from fractures, inversions and translocations. Resende (128) comments that although the theory is not contradicted by any of the data, nevertheless, kalymma is not observable in the salivary gland heterochromatin. I may add that, on the contrary, it is at the extreme proximal heterochromatic end that chromosomes are in contact with the boundaries of sheaths when these are present, as in the monosomes of the Orthoptera.

IV. Functions Attributed to Heterochromatin

Since structure and function are inter-related there is a certain unavoidable overlap between III and IV.

A. Genetic Inertness. The first function assigned to heterochromatin was the negative one of genetic inertness. The present status of this concept was arrived at in a series of steps. Because of the stages through which it has passed, the term "inertness" is not clearly understood in all quarters, and a review of its history is therefore in order.

The Y-chromosome of *Drosophila* has been considered inert for some time (140), as have the supernumerary or B-chromosomes of *Zea mays* (39). By means of translocation and mutation frequency studies, the X-chromosome of *Drosophila melanogaster* was differentiated into a genetically active and a proximal inert region. (110). Dobzhansky's chromosome map of the same species, which appeared the same year (43), localized nearly all the sex-linked genes in the distal 2/3 of the X. Because the proximal 1/3 contained only one gene, bobbed, it was considered inert in the same sense as the Y. Heitz (64, 65), noting that this proximal region is heterochromatic and that it occurs in many *Drosophila* species, suggested that genetic inertness is a property of heterochromatin. The criteria for this inertness are summarized by Darlington and La Cour (38): "... first, our inability to correlate any effect on the forms or functions of animals or plants with the presence or absence of inert parts: secondly, the absence of any spontaneous or induced mutations in these parts..." The problem was posed at the outset (110), whether these inert regions consist of a full complement of genes, most of which are inactivated or degenerated, or whether only a few active genes are present, with much accessory material. The evidence pro and con on this point was reviewed by Muller and Gershenson (105). These authors concluded on the basis of the evidence from breakage, involving inversions and translocations which indicated that a definite part of the inert region accompanied the active gene "bobbed" as a block, that such regions in the Y and X do "not consist of a row of degenerated genes in single file", but rather "of non-genic material derived from a few active genes between which breakage takes place much more readily than between genes in the. . . active region", and that in consequence there is a definite structural difference between eu- and heterochromatin. This hypothesis was strongly supported by the following:

1. the heterochromatin of *Drosophila* salivary gland nuclei has been found to consist of fused terminal heterochromatin;
2. the Y is represented by only a short segment of the salivary gland chromosome, probably the genetically active part;
3. in the salivary gland nucleus the inert region of the X (designated XI) consists of rows of discs spaced at regular intervals, as in euchromatic regions, but the XI and the Y are very short, with relatively few discs and therefore with relatively few active genes.

Later it was established that "bobbed " is separable from the heterochromatin block; nevertheless, the evidence brought forth in this paper indicated the bulk of the inert regions at mitosis to be due to a very few genes. The authors suggested that "the most characteristic property of this region seems to lie in the attraction of its loci for other loci so as to result in the tendency to form a chromocenter", and that "it would be better to term these regions 'chromocentral' regions rather than 'inert regions' ". They affirmed their recognition, however, that these regions do not always form chromocenters [*e.g.*, *Chironomus,* (4, 6)] and that they are genetically inert in the sense of containing few active genes.

Three pieces of contradictory evidence exemplify the confusion in which cytogenetics finds itself in this problem. Hinton (71) showed that the bulk of the heteropycnotic regions adjacent to the centromere in chromosome 2 of *Drosophila melanogaster* are reduced to one band in the heterochromatic region of the salivary gland chromosome, while conversely the bulk of the heterochromatic band in the salivary gland chromosome is constituted from a small euchromatic area distal to the heteropycnotic region in the mitotic chromosome. He refers to this area as "heterochromatin", despite its euchromaticity. Again, it has been stated (52) that, although the inert regions very closely coincide with heterochromatic ones, the proximal 1/3 of the third chromosome in *Drosophila virilis* is also inert, though euchromatic according to Heitz. Again, the heterochromatic X-chromosome in *Sphaerocarpus* produces frequent mutations with marked phenotypic effects (68).

Further evidence for the genetic activity of heterochromatin, particularly in mosses, is cited by Resende (128). He calls attention to the fact that in mosses, Heitz in 1942 (68) demonstrated the inviability of nuclei deprived of even small quantities of heterochromatin.

The concept of the genetic inertness of heterochromatin thus underwent a change from the notion that these regions are chains of non-functional genes which have mutated to inertness because of the heterozygous condition of the chromosome, to the idea of a seriation of blocks of inert accessory material produced by active genes. A parallel modification in the ideas concerning the nature of this inertness took place in another direction:

1. Caspersson and Schultz (22), as will again be noted under *D* below, suggested that the apparent genetically inert and non-specific character of heterochromatin is merely the consequence of its specialization in performing a function also performed by all the other genes, namely, synthesis of nucleic acid.

2. Darlington and La Cour (38) reasoned from the fact that so-called "inert genes" are usually in a constant position on a given chromosome, and from the existence of inert chromosomes in equilibrium in the populations of many species, that they must have selective value and hence some genetic function.

3. Darlington carried this general idea further, and the passage is worth quoting in full: "Dimunition in the individual and equilibrium in the population are therefore two properties of inert chromosomes related to their dependence upon being useful without being indispensable. This condition of usefulness brings us back again to the notion that they are not indeed inert, but rather non-specific in their activity; that the different products do not take place in a series of specific reactions co-ordinated in time and space, with those of different active genes, but rather take part indiscriminately in all gene and cell reactions".

We find this idea of genetic inertness undergoing still further refinement. Mather (94, 95), on purely genetic grounds and to account for the two types of inheritence, one qualitative by sharp phenotypic differences exhibiting variation, and the other quantitative, by small cumulative differences, postulated the existence of two types of gene, *viz.*, the mono- or oligogenes, *i.e.*, the major mutants or qualitative genes which produce significantly measurable changes and give rise to known Mendelian ratios; and the polygenes, producing similar and small more or less additive effects which cannot, as can the former, be individually recognized. The major genes act as "switch genes" in development, sending the organism along one of several possible paths. The polygenes, in collaboration with other gene systems, serve as buffers to "delimit the possible paths with greater or less precision".

Later, Mather (96), citing evidence from genetic experiments with the Y-chromosome, extended this hypothesis with the assumption that the suspected genetical activity of heterochromatin is largely polygenic in its nature. He described polygenic combinations as having two important properties, balanced combinations within chromosomes and a tendency to a certain amount of recombination, thus "combining a high degree of present fitness with the possibility of future change", and concluded that "the observed. . . properties of polygenic combinations exactly reproduce those of the supernumerary chromosomes. The latter are in fact a cytological embodiment of balanced polygenic combinations".

Hence Mather attributes to heterochromatin, as supernumerary chromosomes, and in the Y-chromosome of *Drosophila,* the function of bearing polygene combinations.

The phenotypic expression of polygenes has been described as easily modified by environmental changes or differences

B. Regions of Synthetic and Metabolic Phenomena. Darlington (36) and Caspersson and Schultz, together with their co-workers (135, 136, 22), believe that heterochromatic regions are centers of, or controlling factors in, nucleic acid synthesis—of chromonucleic acid in the chromosomes and of plasmonucleic acid in the nucleolus-organizing region. The ribonucleic acid passes into the cytoplasm where it is concerned with protein metabolism as is evident from its high concentration in growing tissues and tumors. (24, 23). The evidence for this lies:

1. in the frequent topographical relationships between nucleoli and heterochromatin;
2. in the fact that ultra-violet analysis indicates heterochromatic regions to have a high histone content, as does the nucleolus (135, 136, 30,); the assumption is that histones are the precursors of the protein moities of the cytoplasmic nucleic acid;
3. in the fact that in *Solanum* increase in heterochromatic segments produces a corresponding increase in the nucleolus (84);
4. in the fact that in *Drosophila* addition of a Y-chromosome increases the concentration of the ribo-nucleotides in the nucleolus and cytoplasm (22).

Opposing evidence is at hand in the fact that in *Zea* the heterochromatic nucleolus-organizer, when split by radiation into two unequal parts, bears the larger nucleolus on the smaller fragment, and *vice versa* (97). Again, nucleoli appear in nuclei which are very poor in heterochromatin. Fernandes and Serra (51) try to get to the bottom of this contradictory evidence by analyzing the relative effects of euchromatic and heterochromatic supernumeraries in *Narcissus* in increasing the size of the nucleus and the size of the nucleolus. They report that both euchromatin and heterochromatin increase the size of the nucleus proportionally and the nucleolus exponentially, but that heterochromatin is more effective in both cases. Most of the data are explained by assuming that heterochromatin has a more accelerating effect upon metabolism than does euchromatin. The situation in *Zea* remains unexplained but may serve as evidence in a related problem, as will later be seen. A high plasmonucleic acid concentration has been described in the cytoplasm of the *Drosophila* egg (22). In the toad egg the Feulgen-positive granules previously mentioned in connection with the orientation of chromatin detach themselves from the chromosomes and collect toward one end of the germinal vesicle (114). The authors in this case consider such granules to be heterochromatin because of their tendency to associate in clumps and because of their subsequent activity as discrete and independent nucleolar organizers, producing small nucleoli. Eventually they arrange themselves along the periphery of the nucleus, with the granules directed to the outside, the nucleoli within. As strikingly again, the membrane of the germinal vesicle becomes wavy, and the granules with their attached nucleoli occupy the resulting "finger-like pseudopodia".

The situation in *Allium* is of interest here. It may be observed in Fig. 1 of my foregoing paper that the upper nucleolus has a heterochromatic body contiguous to it and to the nuclear membrane. The lower nucleolus presents the same picture, since the larger of its two acolytic granules is actually at the nuclear membrane surface on the upper level. The nucleoli of all cells of the *Allium* root tips are with great frequency, though not always, located near the nuclear surface, and when they are, at least one of the two or more accompanying heterochromatic granules lies between the nucleolus and the membrane, usually touching the latter. A rarer but still not uncommon picture is that of a long thread-like chromatic projection reaching from a more centrally located nucleolus directly to the nuclear periphery. These various configurations are best seen in iron-haematoxylin preparations. In Fuelgen slides the boundaries of the nucleolus are indistinct, and it is difficult to distinguish heterochromatin from euchromatin. The resemblance in the haematoxylin pictures to the final position assumed by the detached nucleoli in the toad's egg, however, is striking. This configuration is also reminiscent of the trophazoite stages of *Plasmodium* where the chromatin granule is often located at the surface of the blood cell.

To continue with the story in the toad's egg, new granules with attached nucleoli continue to arise in the heterochromatic regions of the chromosomes and to migrate to the periphery. Although these do not seem to be extruded into the cytoplasm, Painter and Taylor, using ribonuclease and Unna's stain, confirm Caspersson and Schultz's observations on the sea urchin egg (24) in finding that the cytoplasm just outside the membrane is rich in plasmonucleic acid. Indeed in this egg "there is a very large amount of it with a very sharp gradient, the color being most intense next to the nuclear membrane and shading off toward the cortex". The authors conclude that "the heterochromatin granules and the nucleoli associated with them are part of a mechanism for laying down of ribonucleic acid in the cytoplasm of the toad's or frog's egg".

The above evidence brings into relief the view (136, 25, 132) that the basic cytoplasm of the cell is the product of heterochromatin. The concept is an important one and in need of amplification and elucidation.

C. Transformation of Euchromatic Regions. The property of transforming euchromatic regions, translocated to its vicinity, into heterochromatin is paralleled by the production of definite genetic effects. Genes thus changed to a position near the heterochromatin of the centromere region exhibit a position effect: their phenotypic expression varies somatically in a rather irregular way in various body tissues, giving rise to mosaic types, a phenomenon known in its class effect as "ever-sporting displacement" (108, 109, 134, 111, 22, 126, 132, 113, 41, 42, 56).

From the papers of Schultz (134, 22, 132) the following facts concerning such variegation and its cytological correlations are summarized:

1. Variegated races have chromosomal arrangements involving the heterochromatic regions (22).

2. The breaks involved occur at these heterochromatic regions.

3. The effects are both on the genes in the heterochromatin and on those juxtaposed to it (134).

4. Only those genes whose loci are quite close to the break are affected (132).

5. "The closer the gene to the point of breakage the more extended the variegation, the earlier the development occurs, and the more extreme is the allelomorphism in those cases where allelomorphic series are distinguished" (134).

6. Cytological correlations have been established in some cases. For hypothetical example, where genes *a, b* and *c* have been translocated to a position adjacent to the heterochromatin, with gene *a* nearest to it, gene *a* exhibits the most pronounced variegation, and its corresponding band may be disarranged, even to the point of virtual disappearance. Gene *b* manifests lesser variegation, and its band shows an increased nucleic acid content, appearing darker in ultra-violet and Fuelgen analysis. Gene *c*, with normal phenotypic expression, is correlated with a normal band (134).

7. Breaks which transpose heterochromatin from its normal centromere region remain associated with the chromocenter, notwithstanding. When heterochromatin is inserted between two euchromatic regions, the chromosome forms a loop, with the heterochromatin in its new position still imbedded in the chromocenter, as is the heterochromatic region near the centromere. In the case of Plum 2 heterozygotes "all the broken portions of the chromosomes are joined at the chromocenter" (134, 132).

8. As a converse of 5, removal of variegation genes from proximity to heterochromatin to a locus adjacent to euchromatin restores their expression to normal (134).

From a personal consultation with Schultz on several points which I considered uncertain in the papers from which the above data were taken, I derive that he has altered his view in one or two cases. In his 1936 paper (132) Schultz said "The evidence seems adequate to establish the rule that variegations are associated with abnormal chromosome configurations of the salivary gland chromosome caused in these cases by the aggregation of inert regions to one chromocenter". Since that time he has become more inclined to attribute the effects to the inductive action of the heterochromatin itself, whose non-specific properties are somehow transmitted to euchromatin translocated in its vicinity. In 1939 (134) he stated that in such translocations ". . . an increase in nucleic acid occurs which is greatest close to the heterochromatic area, the greater the nucleic acid content". From 6 above, on the other hand, it is obvious the gradient is not one in nucleic acid content, since the point nearest the heterochromatin which produces the sharpest variegation is most completely disarranged, sometimes to the point of apparent absence, and hence must have a lesser rather than a greater nucleic acid content. It is the next adjacent band, showing more moderate variegation, which shows up darker in ultra-violet and Feulgen techniques, and hence must have the greater nucleic acid content. The bands beyond these being normal in phenotypic expression and cytological appearance, the gradient seems to be one of degree of change in nucleic acid content, rather than in absolute nucleic acid content. Schultz warns that, since many of these conclusions are made from specific observations in specific cases, generalizations should be made with caution. Again, in his 1939 paper (134) he says "To dissociate position effect and gene interaction . . . where the effect of the new translocation by itself is not known, is rather difficult".

Studies on the effect of heterochromatin on the Notch locus in *Drosophila melanogaster* (41, 42) add two more points to the above:

9. Heterochromatin induces unstable changes and "may affect a number of adjacent loci, which effects may be separated from the point of attachment by a number of bands, while euchromatin affects only one locus, close to the break, and in a stable manner".

10. "Different regions of the heterochromatin differ in their potentiality to produce mottling . . . heterochromatin is more effective if attached to the spindle fibre region . . . the nucleolus organizing region has a high potentiality of inducing mottling, but only when it is located in its normal position near the spindle fiber" (41). And again, " . . . quality of the heterochromatic region as well as its relation to the centromere and its quantity are all important factors in determining mottling".

D. Other Functions.

1. Not only does heterochromatin appear to have an effect directly upon genes adjacent to it, but in the phenomenon of variegation it plays a transchromosomal role. Addition of an extra X or Y or arms of the latter to the ordinary complex results in suppression of the mosaicism normally induced by such translocations as have been described (*e.g.*, 132, 133). "In all cases extent of variegation increases as the Y chromosome is lowered" (132). Add to this the data in E and the heretofore mentioned increase in plasmonucleic acid which occurs with addition of a Y-chromosome (22). Schultz summarizes the effects of heterochromatin as changing the nucleic acid content in band of the same chromosome, in bands of other chromosomes, or the amount of substances related to nucleic acid in egg cytoplasm.

In addition to its effect upon the nucleic acid cycle, chromosomal and cytoplasmic, Schultz believes that the heterochromatin of the Y-chromosome may affect the non-chromatin parts of the chromosomes, since he reports an increase in turgor and sharpness of stain with increasing numbers of the Y in different individuals (132). Again, however, in personal conversation he states that the evidence in this case too is insufficient for broad generalizations.

2. Noujdin (113) attributes a trans-individual action to heterochromatin. For one thing he maintains that "one of the peculiarities of heterochromatin is a maternal effect . . . extra heterochromatin of the mother greatly decreases the development of mosaic individuals among those of her offspring that have no extra heterochromatin". He reports finding a paternal effect of this type also, and states that "the two types of influence are inherent in all mosaic forms of the type of eversporting displacement". I cite this work in juxtaposition to that of Schultz and Demerec with the former's personally communicated reservation that it is difficult

to evaluate these findings and the theory of the effects of structural homo- and hetero-zygosity that accompanies them. My own objection is that the English versions are unaccompanied by specific data.

3. Schultz (134) states that "the genes controlling the size of the heterochromatic region of the X at metaphase are located in a few bands of the salivary gland chromosomes (106). The locus seems to be the same as that of the nucleolar organizer (38, 42). According to the work of Neuhaus (112), the situation in the Y-chromosome may be similar, with the large metaphase block proximal to the spindle attachment.

4. A gene has been described controlling the rate of mutation in *Drosophila persimilis,* which depends upon the presence of the heterochromatic Y for its effects; in the absence of the Y, it is without effect. This mutator gene is borne at the distal end of chromosome 2 ((92).

5. A role in neoplasia has been ascribed to heterochromatin. Darlington and Thomas (40) reason that because of the structural changes which heterochromatin is prone to undergo, it might establish malignancy in cells by stimulating recurrent mitosis. Koller (80), on the basis of cytological analysis of numerous neoplasms in a variety of human tissues, concludes that neoplasms are brought on by disturbance in nucleic acid metabolism leading to an excess and that this may be caused by gene mutation or alteration in heterochromatic regions which are specialized in nucleic acid synthesis. The heterochromatic regions in the cells of these tissues (correctly to interpret his term "chromatin nucleoli") diminishes in proportion to the Feulgen-negative nucleoli with development of the neoplasm. Caspersson *et al* (25, 26) believe heterochromatin plays a specific role in carcinogenesis. They find that in the cytoplasm of tumor cells the amount of plasmonucleic acid varies in different parts of the same tumor, and is larger where growth is more rapid. Their assumption with Schultz (23) is that both in this case and in the large amount of ribonucleotides in growing tissue, the heterochromatin elaborates the histones and ribonucleotides of the nucleolus, which pass through the nuclear membrane to the cytoplasm. The problem is, of course, merely part of the broader problem of the role of chromonucleic acid in neoplasia, which has occupied much research and provoked

considerable speculation. The results, which have been adequately summarized by Stowell (141), are contradictory and inconclusive. There is no critical histo- or cyto-chemical evidence sustaining the above conclusion, made from observation, to the effect that an increase in chromonucleic acid occurs as a general rule in tumor tissue, although it does so in some cases. On the other hand, polytene chromosomes are widespread in mammalian carcinoma (11), and such increase would, of course, involve increased nucleic acid content. And one finding which is consistently supported by a growing body of evidence is that nucleic acids and nucleotides have a growth-promoting effect on cells. The literature in this field is reviewed by Stowell (141).

6. Although hardly a function, the view that heterochromatin must play an important part in speciation is entered here (128, 150). The data on heterochromatin as a phenotypic character is reviewed by Resende (128). Although differences in amount and kind of heterochromatin do exist between races and species, this cannot be critical in the broader categories, because both reticulate and euchromocentric types of nucleus are found in the same families (150). But it should be considered, along with the other tools of cytotaxonomy, on the general ground that anything of importance in cell metabolism may ultimately be of value to the systematist.

Resende (128), in view of the fact that not all plants exhibit the negative heteropycnocity, or "nucleic acid starvation" of Darlington and La Cour (37), suggests that the capacity to do so may be correlated with a sort of specialization for surviving low temperatures on the part of plants not ecologically specialized for cold regions. I might remark here that Resende considers Darlington and La Cour's identification of the special segments exhibiting this negative heterochromaticity at metaphase, with the positively heterochromatic chromocenters of resting stage, as purely inferential. In view of the persistence of orientation, however, I do not know what else they could be.

Noting the resemblance of these negatively heterochromatic areas to the constrictions which are not of nucleolar origin, Resende places them with a separate classification which he has set up to delimit these structures from either hetero- or euchromatin—namely, "olistherochromatin", which includes the primary constrictions and secondary constrictions, nucleolar and anucleolar. It will be noted that I have not included primary and secondary constrictions in the list of structures called "heterochromatic," although by strict definition they are regions exhibiting differentials in nucleic acid content and/or cycle. Because they have been considered as constrictions, i.e., as attenuations of chromatin, they have not generally been listed as either eu- or heterochromatin, and Resende's separate category is justified. That Darlington and La Cour's nucleic acid starvation regions should be included with these, however, is a questionable point.

I have thus reviewed the definitions, examples, properties and functions of heterochromatin. The task at hand is their revaluation in the framework of cell topography. Preliminary to this, in turn, is a summation of evidence that the polarity of the cell is reflected in chromosomal structure, for this is the very simplification and essence of the meanings which we have been reading into this topography since the beginning of this paper.

In my preceding paper I pointed out that in five separate groups, the centromere, or its probable homologue, exhibits a regularized association with the nuclear membrane. Such association is deductible from evidence in other forms. It is certainly implicit in the finding of Ribbands (129) that non-homologous centromere-telomere associations occur at the nuclear membrane in the meiotic prophase of *Habrobracon.*

CENTROMERES AND TELOMERES

At this point I shall introduce a piece of evidence of considerable significance to both the problem of cell polarity and the problem of the nature of heterochromatin, namely, that there is some fundamental similarity between centromeres and telomeres. This has long been evident because:

1. Both exhibit a tendency to non-homologous association. Just as centromere regions are united in the chromocenter of the salivary gland nuclei, so the distal ends tend to touch one another, especially in *Chironomus* (4). Also, the small fourth chromosome of *Drosophila melanogaster* sometimes curves upon itself till the distal end touches the chromocenter (132, 107). Examples of non-homologous centromere-telomere association are numerous in the literature.

2. Cross-over values are lowest and most variable in their vicinity (93).

3. Both show high breakage frequency (107).

4. Except at the critical kinetochore region itself, both exhibit relatively high dispensability. Individuals with deletions in these regions show considerable viability (107).

5. Telomeres exhibit the same mosaic position effect found in centromere regions (107).

6. In the reticulate type of plant nucleus, the telomeres, like the centromeres, are often distinguishable as chromocenters, but with distal instead of proximal location. They likewise maintain contact with the nuclear membrane throughout interphase, and though this seems to be more or less obscured in the writhing movements of prometaphase to metaphase, in the formation of the metaphase plate the ends tend to describe in space the outlines of the vanished nuclear membrane. Nothing can be deduced of their location in euchromocentric nuclei or in any forms where there are no distal chromocenters.

7. Stadler (139) and Helwig (69, 70) find that in X-radiation, intercalary breakage ends of chromosomes do not unite with telomeres, while, on the other hand, breakage ends of centromeres do unite with telomeres, and *vice versa.*

"All this leads us to agree with Prokofyeva", remarks Muller in connection with points 1 to 5 above, "that these terminal regions are similar in properties to those normally located near the centromeres" (107).

Centromere regions may be marked by the primary constriction, or by a heterochromatic knob, or by a heterochromatic granule, or by combination of these, as in the case of Doutreligne's euchromocentric nuclei where the heterochromatic blocks lie to either side of the primary constriction.

Turning to the region of the nucleolus, we find a similar range of variety. Secondary constrictions may be present, as described by Heitz, or heterochromatin granules, as in the Orthoptera and the detached nucleoli of the toad's egg. Or combinations of these may exist, as in *Zea.*

The three principal morphologically differentiated regions of the chromosome, the centromere, the telomere and the nucleolus-organizing region, have three properties in common, *viz.,* they lie at or near a membrane surface, they are frequently heterochromatic, and nucleoli may appear in any one of the three regions, according to the species.

With these three topographical landmarks oriented in the cell, the suggestion appears most forcibly that chromosomes are tracks, or lines of euchromatin, connecting the heterochromatic points of juncture with the nuclear membrane and the heterochromatic point of juncture with the nucleolus.

This applies literally only to nucleolus-bearing chromosomes. But, as much indirect evidence seems to indicate and as McClintock has critically demonstrated for *Zea* (97), all the chromosomes of the genome are involved in nucleolus formation; even when the morphological continuity of anastomoses or closely connecting gyres is absent in the resting stage, physiological continuity must be assumed. Chromosomes thus seem to constitute pathways between the exterior cytoplasmic environment and the interiorally located nucleolar environment.

Chromosome polarization is further reflected in two characteristics of the chromonemal spiral. First, the uncoiling in prophase begins distally and proceeds proximally; second, in many forms we find a gradient in gyre size and tightness from the distal to the proximal ends. Gyres are often larger and looser toward the distal, tighter and smaller toward the proximal ends.

How far does this polarization of the chromosomes and of nuclei reflect the polarization of the cell? The latter, of course, consists essentially in the relations the cytoplasmic components bear to the nuclear. Because of the radically different techniques employed, cytologists tend to specialize in one or the other of the two regions, and almost no papers are in existence relating the behavior and distribution of both elements in a single form.

Bowen's illustrations of cytoplasmic elements in plant cells are particularly illuminating (13, 14, 15). In the root tip cells of a wide variety of plants there is a marked tendency for the bulk of the cytoplasmic elements to concentrate at opposite poles. This is exceptionally well shown in citation No. 15, figures 48 and 51, for certain cytoplasmic components of the plerome of *Vicia faba,* and figures 139 to 142 for the plerome of *Pisum.* In these more or less elongate cells, thin borders of cytoplasm with a scattering of cytoplasmic elements lie lateral of the nucleus. The bulk is distributed between the two poles. It will be recalled that this is the same as the distribution of the chromocenters in the *Allium* root tip nucleus.

Unfortunately for the precise character of such correlation, a bipolar distribution of chromocenters is lacking in *Vicia faba;* they are more or less gathered in one half of the cell [see (12), Plate I.] It is difficult, moreover, to determine which are the centromere regions. Distal chromocenters are lacking. But if it be recognized that concentration of nucleic acid in chromocenters may be only one of the activities of centromere and telomere regions, or, conversely, that these regions may be active without production or accumulation of nucleic acid, then the polarity of the cytoplasmic inclusions shown by Bowen may reflect the chromosome polarity evident in the figures of Heitz, since the chromosome ends are there depicted as touching the nuclear membrane at each pole. The shifts undergone by the nucleus during the mitotic cycle do not, until just before prometaphase, depart more than 20 to 25 degrees from this polarity.

PHYSICAL AND CHEMICAL PROPERTIES OF NUCLEIC ACID

In dealing with chromatin it is predominantly the chromonucleic acid with which we are concerned. Let us therefore ask a question. Which of the known physical and chemical properties of nucleic acid serve in any way to account for the conspicuous and widespread characteristic of chromatin mentioned in Number 8? Better yet, let us broaden the base of this question—Are there any properties of chromonucleic acid, physical or chemical, which help explain why it is so indispensable to all forms of life at the cellular level? Do any of these properties account for its pronounced tendency to peripheral distribution in the structures of which it is part?

The English cytologists have stressed one important feature—its difference from plasmonucleic acid. Darlington (36), amplifying the views of Caspersson, states that since the two nucleic acids differ in the central sugar radical of the nucleotide, "the desoxyribose radical apparently . . . gives its nucleotides a flatness to which they apparently owe their capacity for polymerization. They form columns of plates which, as shown by polarized light, lie crosswise to the protein thread. These plates agree in spacing at 334 A with the extended polypeptide chain of the chromosome". To this Koller adds "The polymerization of the thymonucleic acid is responsible for (1) the coiling, (2) the reduplication . . . of the chromosomes, and (3) making them visible". Except for Number 2, this is in contrast with the fluid non-fibrous nature of the non-polymerized plasmonucleic acid. Since the viruses, most of which are ribonucleic in nature, also possess the power of reduplication, polymerization cannot be the cause of chromosome reduplication.

What are the chemical properties common to both types of nucleic acid? They are polynucleotides and possibly tetranucleotides or even poly-tetranucleotides, although the evidence here is somewhat contradictory. Nucleotides consist of a sugar molecule united at one end of its carbon chain with phosphoric acid, and at the other with a purine or pyrimidine base. On alkaline hydrolysis the sugar and purine or pyrimidine sections split off from the phosphoric acid in combination as nucleotides; mild acid hydrolysis splits off the purine or pyrimidine base, leaving the sugar combined as an ester with phosphoric acid. The nucleotides, in common with other proteins, are amphoteric in nature. They contain phosphoric acid which is of great importance because of the role played by phosphate bonds in the cognate and interlocking fields of cellular respiration and carbohydrate metabolism. The nucleotides of nucleic acid contain purine and pyrimidine bases which are vitally and intimately concerned with the oxidation-reduction mechanisms in the cell. Lastly, very significantly, they have the same general structure as the oxidation-reduction enzymes; that is, they are conjugated proteins, consisting of a prosthetic group of low molecular weight joined in loose or rigid combination with proteins.

Thus far this structure has characterized many of the demolases, or respiratory, enzymes. The hydrolases do not seem to require a prosthetic group for their function. The pyridinoprotein and flavoprotein enzymes are mono- and di-nucleotides. The adenylic acid of the cytoplasm, which is so important not only for its role in phosphorylation but also as an oxidation-reduction co-enzyme, is found in plasmo-nucleic acid and differs from the adenine monophosphate of chromonucleic acid only in the sugar radical, and possibly in an isomeric arrangement of the phosphate bonds on the purine base. A cytoplasmic di-adenine phosphate has been isolated in yeast, and it is interesting to note that the linkage between its adenylic acids is very alkali sensitive, as are the internucleotide linkages of nucleic acid (89).

Preliminary to a more detailed discussion of these matters, and for purposes of logical treatment, the skeleton framework of the oxidation-reduction mechanism of cells is recalled to the reader's mind (54, 103). In this system oxidation, which in its essential nature is the removal of electrons, is accomplished by a series of carriers—a chain of oxidizable and reducible substances along which the electrons pass from the substrate until their final appropriation by molecular oxygen. Nearest the molecular oxygen are cytochrome oxidase and the three heme-protein cytochromes; at the other end, in order of nearness to the substrate, are specific dehydrogenases, the coenzymes I and II, which are present universally in cells, and the flavoproteins. There may be various omissions from and additions to this; moreover, the system is not limited to enzymes alone, since various intermediate metabolites may act as oxidation-reduction catalysts, as in the Krebs tricarboxylic acid cycle. However, the cellular respiration sequence may for our present purposes be outlined as follows.

Substrate
↓
Specific dehydrogenases
↓
Pyridine nucleotides
↓
Flavoproteins
↓
Cytochrome system
↓
Cytochrome oxidase
↓
Molecular oxygen

Thus at one end the dehydrogenases oxidize the metabolites, which do not act directly with carriers or oxidases. The dehydrogenase-flavo-pyridinoprotein system is concerned with hydrogen mobilization and transport. At the other end the cytochrome oxidase system mobilizes oxygen, and the system as a whole involves a flow of H and electrons from substrate to the atmospheric oxygen.

The pyridine nucleotides and the flavoproteins are of exceptional importance in this chain. As has been noted, both are compounds of nucleotides joined to proteins by phosphoric acid bonds, with the nucleotide functioning as co-enzyme (or prosthetic group) and the protein as apoenzyme, to employ von Euler's terminology [see Goddard (54)] in which the co-enzyme-apoenzyme system as a whole is known as a "holoenzyme". Specific substrates are usually acted upon by specific dehydrogenases. The pyridine nucleotides and flavoproteins are perhaps universally present in cells. They may act as carriers or as direct dehydrogenases. And although the cytochrome system is usually present, it is the pyridino-flavoprotein complex which is indispensable, since under anaerobic conditions, fermentation occurs through their mediation with the cytochrome system non-functional or wholly lacking.

That the two pyridine nucleotides are derivatives of nicotinic acid is thought to account for the importance of this vitamin. These nucleotides are specifically nicotinamide adenine nucleotides, and it is the nicotinamide ring which undergoes cyclic oxidation and reduction. Co-enzyme I, diphosphopyridine nucleotide, also called "cozymase" and "codehydrogenase I", is a dinucleotide. This may be represented as

$$\text{nicotinamide-ribose} \left\{ \begin{matrix} \text{2 phosphoric} \\ \text{acid molecules} \end{matrix} \right\} \text{ribose-adenine}$$

and coenzyme II, triphosphorpyridine nucleotide, as

$$\text{nicotinamide-ribose} \left\{ \begin{matrix} \text{3 phosphoric} \\ \text{acid molecules} \end{matrix} \right\} \text{ribose-adenine,}$$

Following current usage (138), we shall designate these as DPN and TPN, respectively. DPN and TPN are prosthetic groups for a wide variety of enzymes.

The flavoproteins transfer electrons to the cytochrome complex, either directly or through the mediation of other systems. Unlike the cytochromes, they lack iron and for this reason are cyanide- and carbon-monoxide-insensitive. One prosthetic group of Warburg's old "yellow enzyme" is alloxazine mononucleotide, or riboflavin phosphate.

Alloxazine is a chain of a pyrimidine, a pyrazine and a benzene ring, the pentose alloxazine portion being riboflavin, which accounts for the importance of vitamin-B_2. Alloxazine mononucleotide is also a prosthetic group of cytochrome reductase, a flavoprotein link between the cytochrome and dehydrogenase systems.

Alloxazine adenine dinucleotide is the prosthetic group of a number of flavoprotein enzymes oxidizing co-enzymes I and II.

It is of interest to note that the production of CO_2 and the mobilization of molecular O_2 by electron and hydrogen transport are virtually separate systems; CO_2 is not formed by direct oxidation of carbon by molecular oxygen, but by decarboxylation of the organic acids of intermediate metabolism. The enzymes involved in this system have diphosphothiamine as prosthetic group, with magnesium as co-factor. It is in this connection that vitamin-B_1 is vitally involved in carbohydrate metabolism.

The prosthetic groups of the flavo- and pyridinoproteins as oxidation enzymes thus constitute one group of the cytoplasmic nucleotides. Still another important cytoplasmic nucleotide is adenylic acid. The cardinal importance and the central position in carbohydrate metabolism of the adenosine—adenine mono-, di- and tri-phosphate system may be seen at a glance in the diagrams, p. 208, in Potter (121), and p. 62 in Soskin and Levine (138). This system is thought to occur in all cells. Following current usage we shall designate these as AA, ADP and ATP, respectively.

Adenylic acid, adenine monophosphate nucleotide, serves both as a prosthetic group without intermediate phosphorylation, as when it acts with phosphorylase in the primary phosphorylation of glycogen, and also as a carrier of the energy-rich phosphate bonds in other phosphorylations, de-phosphorylations and transphosphorylations, of which one example is the second phosphorylation reaction of glycolysis, when fructose 6 phosphate is changed to the symmetrical fructose 1, 6 diphosphate.

In the first case the catalytic action of the enzyme is probably enhanced by the binding of prosthetic group to protein by the phosphate bonds. In the second the adenine ribose moiety is a sort of trap for phosphate bond energy which reaches it along oxidation-reduction pathways in glycolytic or oxidative food combustion. As Lippmann (89) points out, "The enzymatic transfer of transphosphorylation is remarkably similar to enzymatic hydrogen transfer".

The picture which is obtained from the literature on phosphate bond energy is one of the $AA \leftrightharpoons ADP \leftrightharpoons ATP$ cycle as the hub of a greater wheel that is the phosphate bond energy cycle as a whole. Its rôle in metabolism may be elucidated by a few preliminary remarks. In the ordinary process of combustion, carbon is oxidized with immediate generation of heat and production of CO_2. In cellular metabolism the situation is different. In fermentation and glycosis the same overall degradation of the initial substrates to the end products occurs, but oxidation and CO_2 formation are, as has been noted, separate processes, and the energy which is released is converted along oxidation-reduction pathways "almost quantitatively into phosphate bond energy" (89). Thus "the various phosphorylations which occur throughout the dissimilation of carbohydrate are the means by which the energy liberated from oxidative steps is prevented from being dissipated as heat and is held or built up for use in endergonic reactions", *i.e.*, reactions which require a supply of chemical bond energy.

According to Lippmann, the phosphate cycle consists of a series of steps. At the lowest level is inorganic phosphate (ph). The first step is its introduction into the ester linkage, containing about 3,000 cal/mol of bond energy(—ph). The second step is the generation of energy-rich phosphate bonds (~ph, 10,000–12,000 cal/mol) over the O/R pathways. Third, these are taken over and distributed by cell catalysts, chiefly adenylic acid. In transphosphorylation reactions the phosphate bonds may shuttle back and forth in the AA. . .ATP system, thus maintaining a high potential level, but the fourth and irreversible step eventually occurs when they fall, first to the ester, and finally, by phosphatase action, to the inorganic level. Lippman continues, "For the undisturbed maintenance of this complicated series of reactions a well-balanced equilibrium is needed between the great number of enzymatic reactions involved. Oxido-reductive formation of ~ph and its removal by adenylic acid must follow in due course, in order to avoid obstruction of the smooth flow or reactions. A finely coordinated interplay between O/R and phosphorylation-dephosphorylation results. This explains why very often adenylic acid functions as apparent co-enzyme of O/R reactions".

The AA $\leftrightarrows$ ADP $\leftrightarrows$ ADT cycle thus occupies a central position in the metabolic turnover. It receives phosphate bonds from sources as 1:3 diphosphoglycorate, phosphopyruvate, acetyl and creatine phosphate, *etc.*, and donates them to glucose, hexose 6 phosphate, pyruvate and creatine. Through the mediation of this cycle the ~ph bonds may (*a*) maintain a high energy potential by transphosphorylations, as when they enter the creatine-creatine phosphate cycle which acts as a sort of v ph reservoir; (*b*) they may be employed in endergonic reactions, such as the synthesis of glycogen; (*c*) they may be added to the vitamins thiamine, riboflavin and nicotinamide to function in O/R reactions and carbon dioxide metabolism; (*d*) they may be utilized in the performance of mechanical work, such as absorption and muscle contraction, and also in conduction and secretion; and (*e*) they may of course enter and leave the AA. . .ATP system itself.

Thus ~ ph bonds, generated on oxidation-reduction pathways, are transported and distributed on the AA. . .ATP system and finally utilized as mechanical work and chemical synthesis. The metabolic phosphate cycle is compared by Lippmann and others to a machine which generates electric current, the ~ ph bonds being "a form of energy utilized for all-round purpose".

The transcellular activity of this system further emphasizes its great importance. Phosphorylation and dephosphorylation are the mechanisms by which in kidney and intestinal absorption glucose is forced across membranes against a concentration gradient. Potter (121) makes the illluminating suggestion that AA. . .ATP may be the common denominator of both intracellular control and transcellular hormonal regulation of carbohydrate metabolism, hence of fat and protein synthesis and, in the last analysis, of growth and differentiation. Integrating with the above is the hypothesis (138) which is not without some direct and indirect evidence, that the various physiological effects of insulin upon carbohydrate metabolism may be due to "a single catalytic influence upon some basic enzyme system". They reason that these effects are fundamentally reducible to "increased rate of entry of glucose . . . from the blood and extracellular fluids into the tissue cells . . .", which is probably produced by hexose phosphorylation through the action of insulin upon the AA. . .ATP system at some unknown locus. Since a rise in blood adenosine and possibly ATP occurs after experimental shock in rabbits, the suggestion has been made that ATP may play a role in shock following tissue asphyxia.

These facts emerge. The cytoplasmic nucleotides are of two types: the flavoprotein nucleotides, catalyzing essential anaerobic oxidation-reduction reactions; and the adenylic acid system, concerned primarily with the transport and distribution of energy-rich

~ph bonds generated along oxidation reduction pathways. The two systems are interdigitating; the prosthetic groups, the pyridines and flavoproteins, are built up by phosphorylation of vitamin and adenine blocks, and adenylic acid itself often functions as a coenzyme in O/R reactions.

Do these current conceptions of the O/R-AA. . .ATP systems throw any light on the problems of the role of chromonucleic acid in gene action and of its tendency to peripheral distribution in the structures of which it is part?

Both plasmo- and chromonucleic acids consist of four polymerized polynucleotides. The cytoplasmic nucleotides are of the ribose, and the nuclear nucleotides of the desoxyribose, type. In addition, they may differ isomerically, that is, in the number of the carbon atom to which the phosphoric acid bonds are attached. So far as our present knowledge goes, there is no uracil nucleotide in the nucleus and no thymine nucleotide in the cytoplasm. The purine compounds, guanine and adenine nucleotide, are common to both. The great importance of the cytoplasmic adenine nucleotides has been discussed. There is evidence that guanine participates in transphosphorylation reactions, and it has been shown to be interchangeable with adenine as a necessary growth substance for lactic acid bacteria, but little else is at present known of its function.

Other purines and pyrimidines are also essential as bacterial growth substances, but otherwise our knowledge of purine and pyrimidine metabolism is largely confined to the destructive production of the end products—urea, uric acid and allantoin. Guanine, adenine, cytosine and thiamine tagged with isotopic N are immediately metabolized to end products and not incorporated in the tissue nucleoproteins. There is no relationship between purines and pyrimidines and the creatine cycle, and no evidence of any transformation of purines into pyrimidines.

Within the limits of our present knowledge, then, the nucleic acid constituents of outstanding importance in cytoplasmic metabolism are adenine, ribose and the phosphate bonds.

Of the pentoses in general, it may be remarked, (*a*) sugars as an energy source would be important constituents in any system concerned with release of energy, (*b*) all monosaccharides are reducing agents, and (*c*) although the hexoses and the trioses are involved in glycolysis and glycogen synthesis, pentoses are not and for this reason may constitute an isolated system of specialized metabolic function.

Of ribose and desoxyribose in particular: (*a*) the rarity of their occurrence is also evidence of their isolation from the commoner types of chemical reaction; (*b*) the ribose sugar in plasmonucleic acid is the reduced form of the desoxyribose in chromonucleic acid, and the two could thus function in an O/R system; the interconvertibility of these two types of acid, as demonstrated by recent findings, seems to suggest that they do; (*c*) their relatively greater instability and reactivity, coupled with their isolation from commoner reactions, would enhance any specialized function they might serve in cell metabolism; (*d*) in DPN and TPN ribose joins the phosphate molecules to adenine and the pyrimidine nicotinamide; in the flavoproteins it unties the alloxazine rings to the phosphate molecules. In AA, ADP and ATP it also joins adenine to the phosphate molecules in ester linkage, which, of course, is the role it shares with desoxyribose in nucleotide structure in general.

The importance of adenine as a growth substance may be accounted for by its participation in the O/R and AA-ATP systems. Here its importance seems to lie in its combination with ribose to form the adenine-ribose nucleotide which serves as a trap or storehouse for the phosphate bonds. As a purine, adenine may be an effective part of an isolated system, serving in a specialized capacity enhanced by its nonparticipation in the molecular turnover of protein and carbohydrate metabolism.

The importance of phosphate bonds has already been shown; they are energy-rich at the ester level, and even more so in the di- and triphosphate combinations. Their breakdown is among the important single-step reactions occurring in cellular metabolism, the attachment to sugar is of significance, since oxidation of simple sugars often involves their intermediate attachment to a phosphate; throughout carbohydrate metabolism we see the close link between oxidation and phosphorylation; and, as has been seen, the phosphoric acid molecules are an important constituent of the cytoplasmic nucleotides which they are thought to bind as prosthetic groups more closely to their protein apoenzymes.

The following seem the salient facts concerning nucleic acids:

1. They have fundamentally the same structure as certain O/R enzymes, in consisting of low molecular weight nucleotides attached as prosthetic groups to proteins.

2. Adenine desoxyribose nucleotide of chromonucleic acid resembles, and the adenine ribose nucleotide is identical with, the adenylic acid which is a carrier in the central cardinally important AA-ADP-ADT system for phosphate transfer and transport.

3. The individual constituents, the pentoses, purines and pyrimidines, do not, so far as our present knowledge goes, participate directly in the molecular turnover of protein and carbohydrate metabolism, as do the amino acids, trioses and hexoses, and even the 4-carbon compounds.

4. The ribose and desoxyribose nucleic acids could constitute or be part of an O/R system, although it has not been critically demonstrated that they do.

In any event the affinities of the nucleotides and their individual constituents are with the known oxidation-reduction phosphorylation systems catalysing and participating in the transactions of carbohydrate metabolism, which are primarily concerned with the production, storage, distribution and release of energy.

Can these considerations throw any light on the role of chromonucleic acid in gene actions and on its peripheral allocation in cell structure?

From Child's lifetime work on physical gradients (27) it may be derived that many cells, particularly those used in experimental work and in the pedagogy of cell structure and division, exhibit two such gradients—a gradient along the anterio-posterior axis running from the more active division pole to the less active distal pole of the cell, and a surface-interior gradient. It is obvious that the distribution of chromatin corresponds to the former gradient; the latter gradient is reflected in the greater peripheral concentrations of chromatin, both in the nucleus as a whole and in the individual chromosomes.

HETEROCHROMATIN CONSIDERED IN THE FRAMEWORK OF CELL POLARITY

In many animal cells the bulk of the inclusions is concentrated at one pole; and in many the chromocenters seem concentrated at one pole. Although no correlations in these pictures have, to my knowledge, yet been worked out, in the grasshopper (unpublished observations) I have been able to establish one critical correlation. Certain slides of the McClung collection of Orthopteran material, in the University of Pennsylvania, chiefly strong Flemming, bring out nuclear and cytoplasmic components with equal force, notably in *Ageneotettix.* At bouquet polarization in the spermatocytes the polar granules are drawn in phalanx near the region where the cytoplasmic components are massed in a mitochrondial cap. As the cap breaks up there is a corresponding break-up and circumferential scattering of the polar granules of the chromosomes. The two movements proceed *pari passu* until both heterochromatin and cytoplasmic components are entirely circumferential.

Much more work needs to be done on these cyto-nuclear relations, but even this scanty evidence seems strongly to hint that chromosome polarity and cell polarity are closely connected, if not identical, phenomena. Would not this be considered as axiomatic by those familiar with the extensive literature on the relations of the centriole to other cell components?

Resuming the thread of the argument, then, in an attempt to clarify the misunderstandings concerning heterochromatin and to reduce the rather diverse array of evidence concerning its structure and function to greater simplicity and order, I have reviewed its examples, properties and functions and have presented evidence of what is probably considered axiomatic, namely that chromosome polarity reflects cell polarity. I shall now proceed to consider the definitions, types, properties and functions of heterochromatin in the framework of polarity.

First on the addenda are the definitions. What heterochromatin is, is a problem complicated by several factors. First, as Schultz has pointed out (134), we have to consider both the protein and the nucleic acid components. Second, as I have been at some pains to show, it is difficult to separate the properties of heterochromatin from the properties of the loci where it is found. Third, the relations between eu- and heterochromatin upon which any definition must be based show great fluctuation.

Taking up the first point, the important fact to bear in mind is that concerning the chemical nature of the protein moiety, we have only one piece of evidence: identification by ultra-violet absorption analysis of the histone nature of heterochromatin and the globulin nature of euchromatin in salivary gland chromosomes (134, 136). And where the chemical distinctions with regard to the nucleic acid components are concerned, we encounter considerable disagreement.

Heitz stated that the Feulgen technique does not distinguish the two types of chromatin (67). He is more recently confirmed in this by Fernandes and Serra (51), but others (37, 31, 128) consider that the Feulgen technique does serve to differentiate the two. My own observations in *Allium* indicate that the differences are much sharper in the iron-haematoxylin techniques than in Feulgen. What is necessary here is the common sense understanding that the Feulgen and ultra-violet techniques reveal only two things, namely, the presence or absence of chromonucleic acid, and the degree of its condensation or dispersion. As a matter of fact, an extremely fine dispersion might easily be reported as absence. When Heitz and others state that Feulgen reveals no chemical distinctions between eu- and heterochromatin, they are right. When Resende and others insist that Feulgen distinguishes beween the two, they are also right because it does show the differences in distribution and amount of nucleic acid by which the two types of chromatin are recognized.

On the second point, if the reader will briefly review sections III E, F, G, H and J, and also IV C and D3, he will confront a number of examples where the properties of heterochromatin are difficult to dissociate from the properties of its loci. Effects are in some cases attributed to the centromeres; in others, to the chromocenter; in still others, to the heterochromatin. Again, in reviewing the similarities between centromeres and telomeres, it must have been obvious that many of their properties as loci were those which had already been ascribed to heterochromatin as substance.

Muller (107) noticed this but failed to exploit the identity because of one piece of evidence. It was found that heterochromatin translocated from the centromere region still maintained its identity. Hence it did not seem to owe its existence to a locus. But the evidence presented by Schultz that such translocated regions remain associated with the salivary gland nucleus chromocenter shows they tend to persist in their function as liaison or contact regions. Thus in any case one common property of heterochromatin, centromeres and telomeres may be specialization for contact with and transfers of energy and/or substance across a membrane. Again, that the loci of specialized activity in these centromere and telomere regions are very specific seems indicated by Prokofyeva's findings, as cited by Painter, that the regions involved in the touching of the distal ends of the salivary gland chromosomes are quite definite, "usually consisting of several faint bands that morphologically resemble those in the neighborhood of the centromeres"; specificity of locus for the nucleogenic region is clear from McClintock's findings that when the heterochromatic nucleolus-organizer is fractured by X-radiation, it is the smaller fragment which bears the larger nucleolus, and *vice versa,* indicating that in this case the amount of heterochromatin is not the important factor, but some specific locus. From the facts it may be deduced that we are dealing with two separate factors: one the properties of centromeres and telomeres as loci; two, the properties of heterochromatin as substance. Two may again be divided into (*a*) the special nature of heterochromatin as liaison material, and (*b*) a quantitative effect of heterochromatin apart from its position effect. In some cases it has been possible to separate these factors (*e.g.,* III E, IV E, 10). The situation may be clarified by the following statements:

1. These nucleogenic regions produce heterochromatin in the limits of their own loci and in adjacent points on the chromosome track.
2. Once such heterochromatin has been produced it tends to persist and may perpetuate itself, at least for a period, as is shown by the evidence from translocations.
3. Centromere regions and heterochromatin both exert an influence, acting at a distance along the chromosome track. The centromere region seems to be more effective, since, as in IV E 10, we find cases where "heterochromatin is more effective if attached to the spindle fiber region".
4. In addition to this position effect, heterochromatin has a quantitative effect in suppressing variegation, which may be comparable to the action of anaesthetics which inhibit in high concentration where they may stimulate in low concentration.

The third point, concerning the fluctuations and uncertainties in euchromatin-heterochromatin relations, may be made clear by a few examples. In prophase the euchromocentric nucleic chromaticity draws over the chromosome like a glove, so that during its progressively distal advance, more and more of the chromosome becomes heterochromatic in relation to the euchromatic region, until the chromosome is entirely pycnotic. At the halfway point there would be no standard at all, and after this, the situation would be reversed.

Again, in the allocyclic changes undergone by the monosome in Orthoptera (unpublished observations) this chromosome as a whole is heterochromatic with respect to the autosomes, since it is in a diffuse state at gonial prophase at the time they are condensed. But at this very time the condensed centromere region is definitely heterochromatic to the rest of the monosome.

The heterochromatic chromosome described in the resting nucleus of *Narcissus* (50) is probably a case like the above. I should interpret the large knob-like region as the proximal end of the chromosome, in touch with the nucleus, and the region displaying diffuseness as one subtending the nucleolus. Here, however, the whole chromosome apparently remains more deeply staining than the other chromosomes, even in the diffuse regions; this author therefore speaks of the whole chromosome as heterochromatic with respect to the others of the set, and of its intrachromosomal differentiation as positive and negative heterochromatin. The designation is self-evidently and inevitably arbitrary, and Resende shows the difficulties into which it leads (128).

Fluctuations in the relations between eu- and heterochromatin under various influences are discussed by Resende (128).

The expansion and contraction in relative size of eu- and heterochromatin has been noted above (II, IV, A). In the meiotic pachytene of *Oenothera,* euchromatin contracts only 1/25th of its length, while heterochromatin contracts 1/2 of its length (77). Add to these cases the fluctuations observable between eu- and heterochromatin under both natural and induced conditions (III H above) and we have a very cogent argument for exercise of caution and specificity in the use of the terms.

With these points in mind, let us review the definitions cited under Section I. It is certainly clear that that given by Heitz (63) and Schultz (136) falls short in not embracing the concept of negative heterochromaticity. Despite the fact that so competent a reviewer as Resende expresses his opinion that allocycly is the sole distinguishing character of heterochromatin (128), it will be clear that as a definition it breaks down in three instances: first, the existence of cases where heterochromatin maintains much the same condition and size relations throughout the mitotic cycle, as seems the case with the alpha chromatin of *Drosophila* (66), second, the cases where a small block of one kind of the two chromatins gives rise to a larger block of the other, or *vice versa;* in such expansions and contractions it would be very difficult to speak of differences in amplitude or intensity of staining; third, the recent finding that heterochromatic regions are still detectable in metaphase, which is presumably the time when the two chromatins would have the same cycle and hence be indistinguishable. The term "allocycly" should hence be clearly distinguished from mere "differential" and should be employed only in such cases as the Orthopteran monosome, where cyclic changes can be traced through several cell generations.

As has been related, the idea of heterochromatin as a special kind of substance characterized by genetic inertness became, and still is, strongly entrenched despite the fact that the Feulgen method does not distinguish between them [(67), p. 409], and that so far as the nucleic acid goes, eu- and heterochromatin are merely varying states of chromatin and not different substances (*ibid,* p. 411); despite, too, all the following evidence: that reversals from one state to another occur under natural and induced conditions; that some heterochromatic regions are known to be active; that, as brought out in III C, the differing aspects in which heterochromatin has been described are paralleled in the varying aspects exhibited by euchromatin itself.

A few examples will illustrate the confusion to which this thinking has led. Resende (128) points out the inconsistencies into which Japha (77) and Darlington and La Cour (37) are led in assuming that the change of state which follows the changes in the mitotic cycle does not involve a shift from heterochromatin to euchromatin. They are thus forced to call regions which are euchromatic in appearance, "heterochromatin". The latter authors believe that species lacking in chromocenters, for instance, actually have the heterochromatin present, but with no detectable allocycly. Hinton (71) similarly designates the non-heteropycnotic section of the somatic chromosome *a* in *Drosophila melanogaster* which gives rise to heterochromatin in the salivary gland, as heterochromatin, although it is certainly euchromatic in appearance. To account for the few active genes in the proximal region of the Y, Heitz (65) postulates that undetectable euchromatic areas may be scattered therein, while, on the other hand, Kaufmann (78), to provide for certain breakage date, and Mather (94), to account for polygene combinations in active gene regions, suggest that undetectable heterochromatic areas may be intercalated in the euchromatin. Thus the only definition which still holds true in the face of an accumulated mass of information concerning heterochromatin is that of Pontecorvo (120). If this be modified slightly, the meaning of heterochromatin becomes confined to its strictly lexicographical sense, namely, a chromosome or chromosome part exhibiting differentials in its nucleic acid cycle from the rest of the chromosome, or the remainder of the set taken as a standard.

An even stricter purism would return us wholly to the word "heteropycnosis", substituting the word "content" for "cycle".

A NEW DEFINITION OF HETEROCHROMATIN

Our next task is to evaluate this definition in terms of the different structures which have been called heterochromatic, and in the light of their relation to cell and chromosome polarity.

Of heterochromatic chromosome parts, it has been shown that chromocenters (both simple and compound), euchromocenters, polar granules, polar caps and the Feulgen-positive granules which Painter reports in the toad oocyte are located near or on the nuclear membrane, while trabants and nucleolus-organizers are associated with or lie near the nucleolus. The puff regions of *Chironomus* salivary gland chromosomes may also be considered as associated with the nucleolar material, as are the bulbs and Balbiani's ring. Thus by far the greater number of structures described as heterochromatic lie on or near the nuclear or nucleolar boundaries. The behavior of the Feulgen-positive nucleolar organizers of *Bufo* in migrating to the periphery of the germinal vesicle, in occupying nuclear protrusions and in orienting themselves towards the outer surface—that is, between their nucleoli and the outer nuclear surface—and the somewhat similar tendencies on the part of the heterochromatin associated with the nucleoli in *Allium,* is striking evidence that this universal property must have some profound significance.

So much for chromosome parts. What of whole heterochromatic chromosomes? The alignment of the sex chromosome, either to the nuclear periphery or to the nucleolus, is so widely known as to require no citations. In *Gossyparis* (131) and *Phenacoccus* (72) the heteropycnotic group of chromosomes is very strikingly peripheral. Likewise the supernumerary heterochromatic chromosome of *Narcissus,* as shown in the edgewise view [(51, Plate III, Figs. 2, 6, 7, 8, 9]. I have examined the limited chromosomes of *Sciara* in Dr. Metz's preparations of *Sciara* testes. Here, too, the same condition appears to obtain, though critical confirmation of this should come from the *Sciara* workers.

The situation in *Zea,* as described by Morgan, was discussed in my previous paper.

As summarized by Dobzhansky (44), in the genus *Drosophila* heterochromatic areas are located principally in the centromere regions and in the sex chromosomes. Should these regions in *Drosophila* conform to what is apparently the general rule that these structures are found at or near the nuclear membrane, further evidence would be at hand for the conclusion that a pronounced tendency exists for heterochromatin, as whole chromosomes or as parts of chromosomes, to be located at or near the nuclear and nucleolar membranes.

The only exceptions with which I am familiar are the megameric chromosomes of Orthoptera (146), (34), the intercalary heterochromatin of the salivary gland chromosomes, and chromomeres where these are interpreted as being heterochromatic. The situation in the first case is somewhat like that of the germinal vesicle stage in the egg, which is one of the few cases where chromosomes lie free in the nucleus, unconnected with the membrane. This is the time of synapsis, and even the monosome of the male Orthopterans is pear-shaped, with its free end often projecting into the interior, and the proximal end attached to the nuclear membrane, as is the case in the looping synapsis of the autosomes, including the megameric homologues. The monosome is, however, much more advanced in its allocycly, shows greater condensation, and, as meiosis proceeds, becomes more and more aligned against the nuclear membrane which it does not desert until metaphase, or shortly before. (Unpublished observations). With regard to the intercalary chromatin of salivary gland chromosomes we do not seem to be on very certain ground. That transformations of euchromatin to heterochromatin take place under both normal and induced conditions has been mentioned. The remark of Poulson and Metz (122) concerning the situation of *Sciara* is pertinent here: ". . . there appears to be no clearcut characteristic to distinguish either the material or the organization of 'heterochromatic' regions from . . . ordinary regions. Rather, there appears to be a series of conditions in different regions, ranging from one extreme to another". Heitz (66) warns that in the salivaries it is difficult at times even to distinguish the chromatic from the alternating achromatic bands.

Nevertheless several authors have described definite intercalary regions in these chromosomes, *e.g.*, Dobzhansky (44). I have examined whole Fuelgen mounts of *Sciara* salivary glands prepared by Dr. Helen Crouse and have observed that where the long trailing chromosomes come into contact with the nuclear membrane, the bands in that region tend to fan out over the surface, giving them a markedly different appearance, similar to that sometimes seen where the sucker-like ends adhere to the membrane surface. The possibility thus exists, and calls for critical investigation, that these intercalary regions may also be points of contiguity or association with the nuclear membrane. When such association is constant, definite areas will have this specific appearance and function. When it is adventitious, variability would be a consequence of the non-specificity of the points of contact. This is only a suggestion, however. Schultz, who is familiar with these cells in the living condition, advises me that he does not think there is any particular association of the chromosomes with the membrane other than contacts throughout their length where they are turgidly appressed against it, which would correspond with the view of Buck and Boche (19). In any event there is nothing in these exceptions seriously to challenge the rule that in most nuclei the conspicuous bulk of the heterochromatin is located at or near the nuclear or nucleolar membranes.

There are several pieces of evidence concerning the degree of this association. At the Rockefeller Institute I have examined Claude's preparations of the liver cells in *Amphiuma.* Here the heterochromatin is perinuclear; when subjected to centrifuging there is a tendency for this to cling to the nuclear membrane, resisting the gravitational force; fixed preparations of centrifuged tissue show the chromatin as a mass at the end of the nucleus directed toward the acting force, and finely drawn out strands of chromatin extend from chromatin masses which cling to the rim of the nuclear membrane; other chromatin adheres to the sides. This can be seen particularly well in Figs. 1 and 2 of Claude's article (28). Resistance to a force of 60 minutes at 1800X gravity argues for considerable intimacy between membrane surface and the contacting chromatin.

As remarked above, the chromosome ends of the Dipteran salivary gland chromosomes likewise frequently give the appearance of being attached like suckers to the nuclear membrane, often fanning out over its surface. On the other hand, the relationship is sometimes contiguous rather than actually contactual. In the condensed stage of its gonial allocycly, the monosome of *Podisma* (Orthoptera) lies at the center of its enclosing vesicle, separated by a clear space from the nuclear membrane, of which the outer wall of the vesicle is part. The chromosome as a whole lies near the periphery, but the chromatic element is separated from it by the achromatic hull of the vesicle (unpublished observations).

The migration of the nucleolus-organizers to the nuclear periphery of the toad oocyte, where they remain near and oriented to the nuclear membrane, is another case in point.

A new definition of heterochromatin may now be formulated, based on all the above evidence. Heterochromatic regions are whole chromosomes or parts of chromosomes, for the most part located on or near the nuclear or nucleolar membranes, which exhibit differentials in their nucleic acid cycle and/or content, from the rest of the genome, or the remainder of the chromosome taken as a standard. This difference may be due, at least in great part, to their more intimate relations with the cytoplasmic and nucleolar environments. Although it occurs most frequently and conspicuously as positive heteropycnocity, or over-condensation, the difference may also be one of greater diffusion and/or lesser staining capacity. Such association may be intimate or loose. It may arise by actual contact with, or nearness of, chromosomes and chromosome parts to the nuclear or nucleolar membranes, or it may be attained by their definite migration to these loci.

Heterochromatin as Liaison Chromatin

The simplest explanation of this topography would be that heterochromatin is in some way concerned with transfers of energy and/or substance across the nuclear and nucleolar membranes.

Exchanges between nucleus and cytoplasm and between chromosomes and nucleolus have always been taken more or less for granted. Recent evidence is more critical and more specific.

(a) During the rapid cleavage incidental to early development in marine eggs, plasmonucleic acid is depleted and chromonucleic acid rises; the cyto-plasmonucleic acid thus seems to serve as a direct source of chromosome material (87).

(b) During the growth of pollen-mother cells in *Rhoeo* the nucleo-cytoplasmic ratio changes from 1 : 2 to 1 : 1, with accompanying endomitotic duplication of chromosomes, and during this time the plasmonucleic acid disappears from the cytoplasm (31).

(c) Macro-incineration studies reveal that in resting stage the calcium and magnesium ash is more or less evenly distributed through the cell, while in prophase this is deposited on the chromosomes (137).

(d) The heterochromatic nucleolus organizers in *Bufo* finally disappear after their nucleoli are formed.

(e) Evidence of a negative nature in support of this view is at hand in the finding that when radiation inhibits growing and differentiating cells, and no chromonucleic acid is being synthesized, plasmonucleotides evidently pile up in the cytoplasm.

Adding to the above data, as recently compiled by Painter (116),

(f) The co-incidence of the location of heterochromatic regions near the nuclear membrane surface where Schultz found the greatest concentration of plasmonucleic acid.

(g) Lillie's older finding that this region shows greater oxidation activity (88).

It may perhaps be quite safely concluded that heterochromatin is a region of transfer of energy and/or substance at the nuclear and nucleolar membranes. This viewpoint was urged in a seminar which I delivered at the University of Pennsylvania, Nov. 29th, 1945. It is an interesting example of the essentially collective nature of all scientific thinking that at that time Painter's remarkably similar views were then in press (116).

On the basis of a cytochemical and developmental study of the interrelations of nurse and gland cells in the bee and in *Drosophila,* Painter concludes: (*a*) that the plasmonucleic acid in gland cell cytoplasm comes in part "from heterochromatin which, through the mediation of the nucleolar apparatus, is converted into . . . components which pass into the cytoplasm to form or help form ribose nucleic acid"; (*b*) that "the nucleolus is a part of an heterochromatin-nucleolar-cytoplasmic nucleic acid mechanism which appears to be essential for the conversion of desoxyribose nucleic acid into the cytoplasmic type"; and (*c*) "that during secretion both types of nucleic acid are used up in protein synthesis and are replaced through the agency of heterochromatic regions which are believed to synthesize chromatin (and possibly the nucleolar protein) which is converted into the ribose nucleic acid needed by the cytoplasm, thus completing the cycle".

Huie in 1897 (74) found that on stimulation by feeding, the gland cells of *Drosera* underwent a dimunition in basophilic cytoplasm, accompanied by an increase in the bulk of the basophilic cytoplasm, accompanied by an increase in the bulk of the basophilic nuclear chromosomes (actually the chromocenters). On restoration of the basophile cytoplasm there was a dimunition of nuclear chromatin and an increase in the amount of the nucleolar chromatin (from the modern viewpoint, an increase in its plasmonucleic acid content). From these and other facts she deduced that "restoration of the cytoplasm is brought about by nucleus absorbing food material, metabolizing it, and then excreting it into the cytoplasm", and thus in a rough way anticipated Painter's heterochromatin-nucleolar-cytoplasmic mechanism.

With certain amplifications Painter's views are essentially the conclusion to which I had come independently in integrating the date of cell topography with genetic and physiological evidence.

(a) My chief additional contribution has been the orientation of this cycle within the framework of cell polarity.

(b) The sensitivity of the heterochromatic region and the increase and decrease in their nucleic acid content in response to external stimuli show that influences are transmitted across the nuclear membrane in the direction of the nucleus. But we cannot rule out the possibility of an exchange of the other directions as well.

(c) As Painter and Caspersson and Schultz have evolved the idea, the cycle is confined to plasmo- and chromonucleic acid. But the individual constituents may also be exchanged—the purines and pyrimidines, the phosphoric acid and sugar molecules, the diffusible components of the protein moieties and associated inorganic ions. Huie may have been closer to the truth in her broader though less exact interpretation. The heterochromatic regions, from their topography and behavior, appear to be regions of exchange of various kinds of energy and substance with the cytoplasmic and nucleolar environments. I should like to propose the term suggested by Dr. Nelsen of the University of Pennsylvania at the conclusion of my 1945 seminar—heterochromatin is "liaison chromatin".

(d) Again, I do not consider this property confined to heterochromatin. The concept, rather, is that heterochromatin, because of its location, maintains a function during the resting stage which may be performed by the whole length of the chromosome during the maximum in its nucleic acid cycle. This is the next point for discussion.

It will be recalled that in the onion-root tip mitosis, as the chromosomes grow more pycnotic in prophase, they tend to associate more and more with the nuclear and nucleolar surfaces until near prometaphase they constitute a series of lines, running from the centromeres near or on the nuclear membrane at the proximal pole to the nucleolus, and from thence on to the telomeres near or on the membrane at the distal pole. Some arms lie free, but the majority of the V's and J's show this alignment.

Again, as was pointed out, this tendency to location near or on the outer surface is not confined to heterochromatin or to heteropycnotic regions; it is a tendency exhibited by chromaticity, or pycnocity, itself—in short, of chromatin in its condensed state at all its levels of integration, as was developed at the commencement of this discussion.

The next conclusion is that the period of maximum condensation and minimum surface area is one specialized for, or at least in some way connected with, transfers of energy and/or substance across the membrane surface or interfaces.

This calls for radical change in our way of thinking about the mitotic cycle and cell structure. The older idea that the diffuse resting stage with its maximum surface distribution is the active metabolic state of chromatin, as opposed to the condensed minimum surface stage, may not be entirely true. The evidence is convincingly for the latter as a time and form of specialized activity, namely, for exchanges of energy and/or substance in definite limited regions. The two states are relative and complementary, just as they are morphologically interchangeable and frequently show varying stages of transition in the limits of a single chromosome.

It is to the great credit of Ribbands (129) that he avoided the error of thinking of heterochromatin in purely genetic terms, and by considering its topographic relations, both in the form he studied and comparatively in the literature, wound up with the answer that "precocious condensation at prophase is correlated with proximity to the nuclear membrane" and suggested that this was because the synthesis of nucleotides, precursors of nucleic acid, occurs at the nuclear membrane". Although this paper appeared in 1941, the point was accorded little attention in the literature.

I have now reconsidered the definitions of heterochromatin and formulated, in terms of the cellular topography of its known structures, and the evidence concerning their relations with the cytoplasmic elements, a tentative modification and extension of Pontecorvo's definition. The next step is to show how great a simplification this definition effects in the complex and often contradictory evidence concerning the properties and functions of heterochromatin.

The Nature of this Definition as an Hypothesis

Heteropycnocity, allocycly and sensitivity to external conditions (III A, B and C) are seen to be quite probably separate yet cognate properties of liaison regions.

Genetic inertness (III D, IV A, B and C) with its corollaries of rarity of spontaneous or induced mutations (III D), and non-specificity (IV B 1), is in all likelihood a consequence of the nucleic acid metabolism which occurs in these regions (III D, IV B 1), involving exchanges of nucleic acid components with the cytoplasm. This point will be amplified in discussing the polygene concept.

The location of histones in these regions of salivary gland chromosomes (III M IV D 2), besides being indicative of a greater simplicity correlated with non-specificity, also fits logically the requirements of low molecular size for transfers across the membrane. The larger globulins are associated with the more distal euchromatin.

If heterochromatin is liaison chromatin, and the centromeres are points of association with the nuclear membrane, then for heterochromatin to be concentrated in the centromere region (III G) would be quite expected. Dobzhansky's view (44) that "concentration of the heterochromatin near the centromeres must be for some reason advantageous", since "inversions in natural populations rarely involve breaks in the proximal sections" is on the wrong track, but may be retained with an epigenetic twist. Heterochromatin occurs here for the same reason that cities occur near the mouths of rivers. The region is a junction.

The same is true of many telomere regions.

Just as large coastal cities tend to transmit their influence to the hinterland, and just as we find a rough gradient in the impact of this influence, diminishing toward the more isolated interior, so the influence of these loci seems to extend inward along the chromosome track. This would explain why in translocations, not the genes in the heterochromatin, which, as we have seen, remains a liaison region even in its new position, but rather, the genes in the euchromatin are affected (IV E 3); it also explains the gradient in effect upon such translocated euchromatin bands (III, IV E 5, 6). These position effects become more understandable in terms of closer relation to cytoplasmic influence.

A similar differential may obtain in the case of the *alpha* and *beta* chromatin described in salivary gland nuclei (III N). This may be a manifestation within the heterochromatin itself, of the same polarized differentiation as exists between heterochromatin and euchromatin—namely, the presence of a more condensed area near the nuclear membrane, with the regions of lesser condensation distad to this. The *alpha* and *beta* chromatin of the heterochromatic chromosome in *Narcissus* (50) also seem a sort of regional gradient, the proximal condensed *alpha* heterochromatin touching the nuclear membrane, the more diffuse distal or central *beta* chromatin subtending the nucleolus.

The difference between permanent and temporary heterochromatin may perhaps be expressions of the difference between constant and adventitious contact or association with the nuclear membrane. The lateral chromocenters which I have seen in *Allium,* but which were not described by Heitz, appear to be places where the convoluted chromosome buckles against the nuclear membrane. In Orthopteran meiosis I have noticed that as the pachytene chromosomes undergo sinuous movements in the nuclear cavity, they are often more intensely chromatic at points touching the membrane; these points of contact appear to arise anywhere on the chromosome. They are not, except incidentally, identical with regions which are normally pycnotic at other stages.

That the centromere or the heterochromatin attachment frequently possesses considerable strength may be deduced from the appearance of clinging to the membrane manifested by the specialized sucker-like ends of the salivary gland chromosomes. In *Amphiuma* the peripheral chromatin adheres to the membrane under powerful centrifugation. A mechanical attachment of some sort would explain

(*a*) some of the cases where chiasmata are not formed in the heterochromatin (IV F),

(*b*) the reduction of cross-over values in the vicinity of centromeres and telomeres (93),

(*c*) Dobzhansky's above-mentioned note (44) that in natural populations, inversions involving the centromere are rare, although frequent in X-radiation; possibly the attachment in this region can be broken only by X-rays which pierce the cell membrane and act upon the unstable heterochromatin; and

(*d*) the tendency for the heterochromatin of endomitotic nuclei to divide later than the euchromatic sections (IV I). In ordinary mitosis the nuclear membrane disappears at metaphase, and the kinetochores become attached to the spindle fibers and are first to draw apart in anaphase, which would, of course, be true also of their proximally associated heterochromatin. In endomitosis the nuclear membrane persists, and if the kinetochores remain associated with it, as we should expect, separation would be more or less of an uncoiling process and might very reasonably take place last in the kinetochore region.

Muller and his associates ask the question, why are there so many peculiar properties crowded together in the centromere region? (106). And since many of the properties they list are among those pointed out by Muller as also characteristic of the telomere regions (107), the question might have been asked of both centromeres and telomeres. To that question I trust this paper will have given some answer.

It should also be recollected that the genes controlling size at the heterochromatic region of the X and probably the Y in *Drosophila* are proximally located (IV, H). The gene controlling mutation is found in the distal end of chromosome 2 (IV, I). In view of what has been deduced concerning the directional nature of influences along the chromosome track, the location at the extremities of the chromosomes or genes which influence other genes may be significant. In polyploid *Melandrium* the genes participating in sex determination and anther development are located in the proximal and distal segments of the Y-chromosome (147). Here we may be dealing with the direct influence of these regions upon the energy metabolism of the cell.

Resuming the thread of the argument, I have been formulating a new definition in terms of various structures which are heterochromatic and their relation to cell polarity. I have shown how the new definition explains a large body of otherwise disconnected facts. Still unconsidered, or incompletely discussed, are (*a*) the property of genetic inertness, or, in latterday terms, genetic nonspecificity; (*b*) the relations of heterochromatin to metabolism, especially in variegation and neoplasia; (*c*) the nature of spiralization in heterochromatic regions; and (*d*) the property of nonhomologous associations.

Nucleic Acid as Concerned with Energy-Metabolism

By way of summation, the following seem to indicate that heterochromatic areas are regions of exchange of energy and/or substance with the cytoplasmic components:

(*a*) The constant association of the heterochromatic centromere and often telomere regions with the nuclear membrane.

(*b*) Their sensitivity to external stimuli.

(*c*) Their noted correlations in position and movement with the cytoplasmic elements.

(*d*) The fact that in many cells the heterochromatic regions reflect the polarity of the cell. The larger proximal chromocenters often appear at the active division pole; the smaller less numerous distal chromocenters at the opposite, presumably less active pole.

(*e*) Demonstration of the plasmo-chromonucleic acid cycle in conjunction with the known concentration of plasmonucleic acid immediately around the nuclear membrane.

(*f*) The proven correlations between the amount of heterochromatin in the nucleus and (*a*) nuclear and nucleolar size and (*b*) concentration of nucleotides in the nucleolus and cytoplasm.

(*g*) The formation of nucleoli at heterochromatic loci.

(*h*) The large body of miscellaneous data concerning heterochromatin which this assumption correlates and clarifies.

Since chromatin in the condensed state shows similar behavior and distribution, it seems likely that some of the functions of heterochromatin are performed by all chromatin at the maximum stage of its nucleic acid cycle.

The following, then, is the proposed working definition in its final form: Heterochromatic regions are whole chromosomes or parts of chromosomes, for the most part located on or near the nuclear and nucleolar membranes, which exhibit a differential in the nucleic acid cycle or content from the rest of the set of the remainder of the chromosome taken as a standard. These differentials may be due to intimate relationship with the cytoplasmic or nucleolar milieux, and occur most frequently as positive heteropycnocity (over-condensation) and with lesser frequency as negative heteropycnocity (under-condensation). In the former state heterochromatin is usually indistinguishable in appearance, topography or behavior from any other chromatin in its maximum stage of condensation; heterochromatin is liaison chromatin.

In view of the fact that the term "heterochromatin" is so well established in the literature, and also, of the necessity for a terminology to reflect the existence of the two different modifications of chromatin, it does not seem advisable to revert to the prior term "heteropycnocity" which lacks the term "euchromatin" as an antithesis.

This definition of heterochromatin is in reality one half of an hypothesis concerning the relations between cell polarity and cell dynamics. The second half concerns the nature of the transfers between nucleus and cytoplasm through mediation of heterochromatin and the function of nucleic acid. Here a certain amount of speculation is necessary. Despite the great advances in enzymology and cell chemistry, our knowledge of actual intracellular processes is still tentative and very sketchy.

To points *a-h,* a few paragraphs above, let us add the following items which have been developed in the course of this discussion:

(*i*) The region in the vicinity of the nucleus, which in some forms has been marked by concentrations of plasmonucleic acid, has also been shown elsewhere to be one of greater oxidation activity.

(*j*) Nucleotides are known to be growth-promoting substances.

(*k*) The plasmonucleotides are especially abundant in rapidly growing tissue, *e.g.,* meristematic, embryonic and neoplastic tissues; so is chromonucleic acid.

(*l*) Depletion of plasmonucleic acid occurs during a period of rapid growth and is accompanied by an increase in the amount of chromonucleic acid.

(*m*) The components of nucleic acid are also important components of the O/R–AA. . .ATP systems.

(*n*) The pentoses, purines and pyrimidines which are found in nucleic acid and the O/R–AA. . .ATP systems do not participate in the general metabolic turnover and may thus constitute or be part of an isolated system of specialized function.

(*o*) The O/R–AA. . .ATP system is thought to be present in all types of cell; the AA. . .ATP system and the phosphate bonds are of central importance in energy metabolism.

(*p*) The two systems interdigitate closely; phosphate bond energy is known to be generated along O/R pathways, and enzymatic transfers of phosphate bonds in transphosphorylations resemble the enzymatic transfers of H ions and electrons in O/R reactions.

(*q*) In the salivary gland chromosomes alkaline phosphatase is located on the Feulgen-positive bands, as though some mechanism were in existence to effect the release of nucleic acid components when and where they are needed.

(*r*) Chromonucleic acid occurs at the surface of the structures of which it is part—at the surface of vacuoles, to the periphery of the chromosomal matrix, and at the nuclear nucleolar membranes.

(*s*) Chromosomes, and the chromatin reticulum, from their behavior and topography appear to be lines of transfer between the nucleolus and the nuclear membrane, with heterochromatin located at the points of junction.

In reviewing the literature on heterochromatin I have been in search of some plausible explanation why chromatin displays this pronounced tendency to peripheral allocation. Now, in clear recognition of their purely tentative and hypothetical nature, I submit that the following series of assumptions would explain the concatenation of facts and current beliefs from *a–s*.

(*a*) The nucleic acids are basically concerned with the energy metabolism of the cell, either in furnishing precursors of the O/R–AA. . .ATP systems or in the function of their constituents as O/R and phosphorylation enzymes or both.

(*b*) The disposition of chromonucleic acid to the exterior of the manifold and diverse structures of which it is part may be due to its orientation to molecular O_2 or to its ultimate source, or to regions of greater O/R and AA. . . ATP activity.

(*c*) The exchanges of energy and substance which have been postulated for heterochromatin from the evidence in points *a–h* above may be due to its supplying certain precursors of the flavoprotein and pyridinoprotein enzymes, and the AA. . .ATP system (the purines, pyrimidines, the pentoses and the phosphoric acid molecules) or to its receiving such precursors from the surrounding medium, or to both.

The contention that chromonucleic acid may itself perform a function in intranuclear and O/R or phosphorylation reactions is contrary to present belief. Painter refers to the chromonucleotides as inactivated enzymes (115). But, aside from the absence from the nucleus of the essential mechanisms of oxidation in the flavo- and pyridinoprotein enzymes, namely alloxazine and the pyridine rings, there is no direct proof for this assumption. For one thing, the chromonucleic acid equivalent of adenylic acid inside the nucleus could function as a carrier enzyme in phosphorylation-oxidation reactions. It does not seem to me that the negative considerations stand very strongly in the way of an assumption which would go far to explain the integrated and interconnecting body of facts and currently accepted beliefs listed above.

In discussing the theoretical problems of protein reduplication in chromosome reproduction and gene reduplication, Gulick (59) stresses that the formation of peptide linkage between amino acids is an endothermic reaction which would require energy from an outside source. He suggests that the necessary peptide linkage might be effected by an exothermic oxidative dehydrogenation of amino aldehyde, or by phosphoric synthesis, which may take place without addition of energy from the outside. Under the interpretation in this paper nucleic acid could have a role in either process.

The argument may be restated. The chief chemical affinities of chromonucleic acid are with the O/R enzymes and the AA. . .ATP system. The consistent orientation to the surfaces of structures of which it is part may be due (*a*) to the intranuclear function of chromonucleic acid as an O/R-transphosphorylation agency, or (*b*) to the fact that it is supplying certain precursors of the flavoprotein and pyridine enzymes and the AA. . .ATP systems, including those common denominators, the phosphate bonds; or, (*c*) it may be receiving such precursors from the surrounding milieu; or (*d*) any combination of the above functions may be occurring, simultaneously or cyclically.

One over-all cycle has been established with some certainty, and that is the plasmochromonucleic acid shuttle. Plasmonucleic acid is apparently converted across the nuclear membrane to chromonucleic acid, and at the nucleolus, or across its membrane, back to plasmonucleic acid, which is in one way or another returned to the cytoplasm.

The heterochromatin-nucleolus-ribose nucleic acid mechanism of Caspersson and Painter, oriented in terms of cell polarity, may perhaps be clarified as a circuit in space—at the centromere and possibly telomere regions there is a change from plasmo- to chromonucleic acid. The heterochromatin in these regions may best be interpreted as an index of this and other related activity.

I have stated that on the ground of their topography and behavior, chromosomes seem to function as pathways of transfer of energy and/or substance between the cytoplasm and nucleolus. One of the possibilities we should consider is that conversion of plasmo- to chromonucleic acid at the heterochromatic centromere and possibly telomere loci is in one aspect, at least, an oxidation, and since the reverse conversion from chromo- to plasmonucleic acid is a reduction, such transfer may conceivably itself be an oxidation-reduction system, occurring in a chain of H and electron transfer along the length of the chromonemata.

Again, the distribution of AA. . .ATP and phosphate molecules, and of the phosphatase enzymes (35, 83) all along the length of this tract (in the salivary gland chromosome), at least permits the supposition that phosphate bond transfer can also thus be taking place, when it is remembered how integrated O/R and phosphorylation systems are.

The function of chromonemata as chains of energy transfer would fit in well with the mechanical and chemical requirements of Janssen's ingenious hypothesis (83). In this the polymerized nucleotide with its adjunct proteins serves both as a mold and as a synthetic mechanism in which the polypeptide chain is produced by "a wave of oxidation, reduction and condensation". This hypothesis is in the realm of pure theory, but, as Gulick comments, "It is particularly interesting that Janssen envisages the systematic production of a protein (nucleic acid) mold". The work of Avery and his associates (1) seems to indicate that pure chromonucleic acid, freed of all associated substances, can preside over the synthesis of polysaccharides. Polysaccharide synthesis requires $\sim$ph bonds, and when these are present, energy does not have to be supplied (59). This little constellation of mingled truth and conjecture contains one highly important inference—the nucleic acids may be the only substances in the organic kingdom which can both engineer the synthesis of the larger molecules and at the same time provide the necessary energy.

Still, at the time of writing, protein synthesis by pure nucleic acid has not been demonstrated, and it seems a little incredible in the framework of our present knowledge that a compound so relatively standard to all forms can be responsible for creating the infinite variety, complexity, and cell and species specificity of proteins. If it is the responsible agent, then we must somehow attribute its versatility in synthesis to stereoisomerism. At the present stage this is a very generalized supposition, lacking reenforcement by any plausibly detailed theory such as Janssen's.

For the present, then, I shall continue with the thesis that the relative isolation of the nucleic acid constituents, the pentoses, the purines and the pyrimidines, from the turnover of protein and fat metabolism, argues for their dedication to a specific purpose, and that chromonucleic acid in the nucleus may be chiefly concerned, as seems likely for the plasmonucleic acid of the cytoplasm, with the transactions of energy metabolism.

From this comes a further elucidation of the possible role of heterochromatin. As a condensation of nucleic acid near an interface its function in regulating protein metabolism may be due to a more direct control of the intermediary energy metabolism—that is, over the wheels which drive the factory rather than with the direct processing itself. Within the lifetime of the cell this function would naturally never completely be suspended. The great importance of the role of the nuclear membrane in mitosis, which it has been one of my tasks to emphasize, may be due to the fact that energy transfers are then taking place which are part of the complicated and little understood mitotic mechanism. The condensation of chromosomes at metaphase may be directly correlated with the energy changes which must be taking place in their at least partly autonomous movement to the poles. Autonomous chromosome movements have recently been reviewed with new examples by Hughes-Schrader (73). Phosphate bond transfer, which is known to furnish the energy for muscle contraction and the transport of glucose across organ membranes, may be the important energy source in chromosome movements during mitosis. Krugelis (83) advances this suggestion. In discussing the action of alkaline phosphatase localized on chromosomes, she remarks, "while it is also obvious that catalysis of the synthesis of mononucleotide by phosphomonesterase is an essential step in nucleic acid synthesis, one's attention . . . tends to focus on the possibility of dephosphorylation of nucleotide as a source for nuclear and cytoplasmic reactions . . . the actual availability of energy for such cyclic nuclear mechanical processes as chromosome coiling and mitotic movements may be dependent upon a cycle of binding and release of mononucleotide from its nucleic acid storehouse".

In the present paper I have proposed a new definition of heterochromatin, namely, that it is a differentiation in the nucleic acid cycle and/or content, usually owing to interface location. I have tried to show that its consistent tendency to peripheral location is also shared by most chromatin at the maximum stage in condensation. When it is recognized that such a state or stage is in some way concerned with transfers of energy and/or substance across a membrane, we can find sound common sense basis for the affinities between nucleic acid and the O/R–AA. . .ATP systems.

The Nature of Enzymes as Related to the Problem of Heterochromatin—the Polygene Concept

By way of transition to the next point I should like to stress three aspects of our present views concerning the nature of enzymes. First, "They appear to be definitely localized in the cell" (103); in many cases they are known to be identical, or closely associated with definite cell structures. The relationship between myosin and adenosine triphosphatase is a case in point (48, 49, 144). There are many others. Green (57) is even willing to admit visual purple as an enzyme because it is a conjugated protein with vitamin-A as a prosthetic group. Second, "Cell structure plays a major role in the orientation of cellular metabolic systems, particularly those concerned with oxidation" (32). Third, "The concept of what an enzyme is has in recent years been very greatly extended". Green (57) holds that "any protein which uniquely performs some specialized function should earn the title of enzyme", and he believes that hormone activity will eventually be shown to be enzymic in nature, in the same way as vitamin activity. Such an interpretation brings in carriers and co-enzymes. The inorganic ions, such as Ca++, Zn++ and Cu++, are known co-factors or adjuvants of enzymes; therefore the possibility suggests itself of thinking in terms of the enzyme system as a whole, including not only proteins, but all catalytically functioning trace elements. Third, the idea that genes and enzymes are related and in cases even identical has long been entertained. The classical megamolecular autosynthetic nucleoprotein gene consists of conjugated proteins with a prosthetic group. So do the O/R enzymes. If in frank speculation we carry over the extended viewpoint which has developed in the field of enzymology to the still purely speculative field of physiological genetics, we may effect a very useful synthesis.

At the purely genetic level of investigation it was concluded by Darlington and others, from the fact that the so-called inert genes are not indispensable but are at the same time useful, that their activity is non-specific, taking place "indiscriminately in all gene and cell reactions" rather than "in a series of specific reactions co-ordinated in time and space", as in the case with the detectable active Mendelian genes. This view is basic to Mather's polygene concept. In this interpretation genes are of two types, the active saltational oligogenes producing specific discontinuous and readily detectable phenotypic effects, and exhibiting Mendelian ratios, and the non-specific polygenes which produce small similar additive effects and hence are individually non-detectable, and give rise to discontinuous variation. The former, Mather associates with euchromatin. The latter would be located in those regions involved in energy transfers across an interface.

If the concept of a polygene can be extended to include the phosphate bonds and the nucleotide elements concerned in O/R systems, the reason for the cumulative nature, the non-specificity and the undetectable nature of these polygenes becomes clear at once. They may be compounds involved in the energy transactions of the cell, which are chiefly assumed by intermediate carbohydrate metabolism. Such elements would necessarily be non-specific, undetectable, cumulative in effect and productive of continuous variation. They would play the same role in cellular metabolism that the energy sources play in a manufacturing process. The active oligogenes, on the other hand, may be those which produce specific alterations in protein synthesis perceptible as marked alterations in the phenotype. They would then be comparable to agencies effecting changes in the processing machinery itself.

To repeat, if we broaden our concept of the gene, as the enzyme concept may be broadened, then the various compounds which may enter the O/R-AA. . .ATP systems across the membrane at the loci where the chromosomes lie near or in contact with it, may be genes in their own right. The location of the gene adenineless very close to the centromere region in three chromosomes of the tentative *Neurospora* map (8) may be part of this picture. As pointed out above, the centromere regions in these chromosomes are located at the apex of a pear-shaped nucleus; that is, they are most likely on or near the nuclear periphery. Granting that this has the significance that this gene is located where the exchange of AA. . .ATP precursors would be likely to occur, then the gene for adenineless might turn out to be nothing more complicated than the absence of adenine in effective quantities, and conversely, the gene for adenine, the presence of effective quantities of adenine itself.

I am not proposing so radical an extension of our views, but rather, proposing its discussion and pointing out that if polygenes should prove to be varying quantities of individual molecules, the spectrum of the gene concept may range from the infinitesimal of ion-catalysts or co-factors, through phosphate molecules, through the purine-pyrimidine, nucleoside, nucleotide hierarchies (*e.g.*, through adenine, adenosine, and adenylic acid), with incrementally greater effects to the conventional autosynthetic protein megamolecules which produce visible phenotypic changes.

In any case, however, the following assumption appears reasonable. The regions in the neighborhood of the centromere which are presumably liaison regions, and the heterochromatic chromosomes, which have been found to lie on or near the nuclear membrane, are largely polygenic in genetic make-up; it may be that polygenes are for the most part concerned with the catalysts and enzymes of the O/R—phosphorylation systems. The euchromatic regions, on the other hand, are predominantly occupied by saltational genes which presumably exercise control over enzyme systems directly involved in the more specific processes of protein synthesis.

This assumption would explain a number of the functions and characteristics of heterochromatin which have thus far remained undiscussed or incompletely treated. The non-specificity of heterochromatin may have several different aspects: (*a*) it could be due to the nature of heterochromatin as a region of "specialization in performing a function also performed by all the other genes, namely, nucleic acid synthesis" (IV B, 1); (*b*) a very few active saltational genes are located in this region; the important substance seems to be chromonucleic acid itself rather than the protein molecule to which oligogene specificity and individuality may be owing; (*c*) the polygenes of this region may actually be the nucleic acid precursors of the O/R-phosphorylation systems in the cytoplasm, and their non-specific cumulative nature due to their role in energy metabolism, the general patterns and substances of which are common to all cells and the effects of which would be more quantitative than qualitative. Thus non-specificity, reduplication (IV, A), greater simplicity (lesser differentiation) and having an architecture of similar units (IV, M) may all be different aspects of chromonucleic acid synthesis, concentration and greater importance to cytoplasmic metabolism at these liaison regions.

The increase of nuclear and nucleolar size with increased amount of heterochromatin may be a consequence of this role, while the accelerating influence of metabolism and its greater effects on nuclear and nucleolar size than euchromatin may be due to both this same role in energy metabolism and also to a more strategical location near the cyto- and nucleoplasm. The role of heterochromatin in protein synthesis may be essentially intermediary through a more pronounced influence upon carbohydrate metabolism; moreover, that the basic cytoplasm is a product of heterochromatin would only indirectly be true, in much the same sense that processed goods are products of the coal-pile consumed in their manufacture.

The concentration of plasmonucleotides around the nuclear membranes and the link between nucleic acid components and the respiratory enzymes as I have noted, checks with an older finding of Lillie (88) that the region around the nuclear membrane is a region of greater oxidation activity.

Throughout the literature on the effects of heterochromatin on variegation there is a tendency to explain certain facts on the basis of its relative proportions to euchromatin. If heterochromatin has an accelerating effect on metabolism in comparison to euchromatin, then changes in their relative proportions during development may produce differentials in rate of gene and/or enzyme action, with resulting phenotypic mottling. The basis for this is suggested by the fact established in IV D 4. Gulick (59) suggests the possibility that heterochromatin contains pace-making genes which control the rate of activity of other genes. The view advanced here is more direct, namely, the possible identification of the changes of pace with changes in quantity of O/R phosphorylation precursors, and the possible identification of the genes responsible (polygenes) with the precursors themselves, or combinations of such precursors.

Heterochromatin and Neoplasia

This last point leads directly to a major problem in the role of heterochromatin in neoplasia. In his ideas above, Gulick reflects the classical genetic viewpoint and invokes the presence of controlling genes to account for the effects of heterochromatin. The definition of liaison heterochromatin presents another, and from a dynamic standpoint, more plausible alternative. The sensitivity of polygenic systems to environmental change (IV, C) is probably owing to a combination of direct sensitivity of cytoplasmic energy metabolism to external conditions and the sensitivity of heterochromatic regions themselves to environmental change. In brief, these may be regions where epigenetic rather than the classically genetic influences are in operation.

Koller (80) attributes malignancy to quantitative changes in nucleic acid synthesis leading to disturbance of the hetero-euchromatin balance, which in turn causes increased division rate. But it will be evident when heterochromatin regions are oriented in space and viewed as liaison regions with nucleolus and cytoplasm that we are dealing with a circuit, and to pin responsibility for malignancy down to any one point on the cytoplasm-chromocenter-heterochromatin-euchromatin-nucleolar heterochromatin-nucleolus circuit would involve more extensive research and more exact methods than have yet been possible. I submit for consideration by those more competent than I to discuss it, the following per-over-simplified view concerning neoplasia:

(1) The common denominator in malignancy seems to be an abnormal rate of cell division.

(2) In all malignant cells whose metabolism has been studied there is an increased amount of glycolysis (121).

(3) Malignancy is accompanied by definite observable changes in the nucleolus and heterochromatin, and often by an increase in the amount of nuclear chromatin (141, 80).

These facts seem to add up to the fundamental cause as an interference with the normal energy metabolism of the cell as it is regulated by the organism as a whole. But the evidence does not yet warrant our saying at what point this interference occurs or is most effective. In other words, a disturbance in energy-metabolism in the cytoplasm of malignant cells may be just as much a causative or controlling factor as the abnormal conditions existing within the nucleus.

I shall postpone a discussion of spiralization in heterochromatin to the series of papers on Orthopteran spermatogenesis which will follow this paper.

Non-homologous Associations

The remaining peculiarity of heterochromatin—its tendency to non-homologous association—cannot be explained by any of the evidence which has here been assembled. There are many plausible explanations other than the postulated conjugation of similar genes:

(*a*) These regions may be transition areas between polymerized and non-polymerized states which would exhibit greater stickiness.

(*b*) They may be regions where relations with the surfaces abolish charges which normally cause repulsion between non-homologous chromosomes.

(*c*) They may be regions where the chromosomal sheath is absent or very reduced, exposing the nucleic acid elements so that they tend to associate when in contact.

(*d*) If, as Ribbands has shown, the affinity is not due to heterochromatin as such, but to centromere and telomere loci, and centromeres and telomeres are in rapport with the cytoplasmic environment, then such associations may conceivably be due to their joint relations with a common cytoplasmic influence. The intimate relation between heterochromatic regions and the cytoplasmic components in the germ cells of many animals illustrates this possibility.

No really critical evidence is at hand for electing any one or any combination of these possible reasons. Investigations both critically specific and broadly comparative will be necessary to my final answer.

Yet I have the feeling that (*d*) is nearest the answer and that isolation and concentration on one or two characters may obscure a higher truth that has long been hidden in a forest of detail. Non-homologous associations are merely part of a chain of structures which reflect the particular way the cell conducts its energy relations. Stated in another way, these associations are integrated and regulated by the particular energy system of the cell itself.

Thus in *Drosophila* the heterochromatic regions, which may be reservoirs and distributing centers of phosphate bonds and the precursors of the O/R-AA. . .ATP systems, are like so many banks located in one large city, the chromocenter. The three-dimensional picture of this cell is usually obscured by smearing techniques. It may be compared to a large spider encased in a glass sphere, suspended by its body from the upper inner surface. The eccentric chromocenter resembles the body from which the autosomal chromosomes radiate like legs. According to Emmens (47), these are applied closely to the nuclear membrane, with some coiling into the center. On the other hand, according to Buck and Boche (19), in the living condition they fill the nucleus completely, so that the exterior boundaries appear turgidly appressed against the membrane. Emmens' conclusions were drawn from iron haematoxylin sections which, despite their more radical distortion of the cell, may serve better to reveal connections between structures. Underneath the nucleus, like the egg sac of a wolf spider carried beneath the body, is the nucleolus, attached to the small sex chromosomes as an egg sac might be held by specialized palps. In *Chironomus* and *Sciara,* on the other hand, the liaison regions are separate; to revert to the preceding comparison, the member banks are located in different places, rather than in one metropolis. We find the same variations in the plant kingdom, from individual chromocenters to varying agglomerations of these, as in *Collinsia* (63). All are modifications of a basic type of organization. It would be of interest, by intensive localization studies in the cytoplasm of these differing types, to see whether there is any corresponding difference in the topography of the cytoplasmic elements.

SUMMARY

The Cell as an Energy Transformer

I have now completed my task of reviewing the various definitions of heterochromatin in the light of its distribution in the cell, and its relation to cell and chromosome polariy. I have progressively altered Pontecorvo's definition to one implying the nature of heterochromatin as "liaison chromatin", the term suggested by Dr. Nelsen. And I have shown how the concept may unify and clarify an otherwise heterogeneous and disconnected body of details concerning its properties and functions.

Throughout I have found a theme emerging which, as a pure morphological cytologist, I only imperfectly discerned when this study was first undertaken—the simple underlying nature of the cell as an energy transformer. Of this I had had glimpses, however, and this paper in its original form, as submitted to Dr. Metz in 1942, stressed the nature of heterochromatic structures as liaison regions, and of chromosomes as pathways for the transfer of energy and substance. What emerges most vividly is the probability that, had we gained our knowledge of the cell at the molecular level, and could not view the higher integrations with which cytology is concerned, nevertheless it might be possible to predict from the properties of nucleotides and nucleic acids, of what sort of structures they would form a part, and what functions they would perform. Because the nucleotides and phosphoric acid are parts of the O/R-phosphorylation systems; because chromonucleic acid has the property of polymerization; because it is presumably a prosthetic group of megamolecular genes; because both nucleic acids are part of the autosynthetic virus nucleoproteins; and because of the critical evidence of Avery *et al.* (1) that pure nucleic acid is at least capable of carbohydrate synthesis, it might be deduced *a priori* that chromonucleic acid could take part in structures possessing combinations of the following functions:

(*a*) Participation in energy metabolism through its components acting as catalysts or precursors of catalysts,
    (1) in cytoplasmic metabolism
    (2) in intranuclear autosynthetic and gene/enzyme activity.

(*b*) Service as pathways for the distribution of energy and substance, such as electron and H transfer. Under this head there are other possibilities. See, for instance, the generalized speculations on interatomic and intramolecular systems advanced by Bateman in Hober's symposium (3); and also the suggestions of Szent Györgyi (143).

(*c*) Participation in gene structure and action
    (1) With nucleic acid as prosthetic group
    (2) With nucleic acid performing the actual synthesis (Janssen's hypothesis).
    (3) Through its function in energy metabolism as in *b*(1).

Bateman (3) advises us "to discern in the specificity of collodial systems the emergence of new qualities which depend for their very existence upon an intrinsic tendency of matter to become oriented both in the geometrical or morphological sense and in the sense of a correlated localization of energy transfer". The possibility that the cell is an expression at the microscopic level of this tendency of integration is basic to the present hypothesis.

Assuming that future researches do confirm the main tenets of this hypothesis, what is its value? For one thing, it is a unifying principle. As was stated in the introduction, the various disciplines have been tunnelling separately into the problem of cell structure and physiology. This assumption unites and makes more useful a number of their separate findings. Child's antero-posterior metabolic gradient and surface-interior gradients in the cell would parallel the morphologic differentiations implied in the term "cell polarity". The difference between heterochromatin and euchromatin, and between polygenes and oligogenes, would be oriented in this framework, as would Painter's cytoplasm—heterochromatin—nucleolus—cytoplasm cycle. The established link between the O/R enzymes and vitamins is helpfully reenforced by the existence of a link between nucleic acid and the O/-phosphorylation mechanism. This would tie in with the circumnuclear distribution of ribonucleic acid, the perinuclear distribution of heterochromatin, and the polar differences in chromocenter size and number; a very useful pattern would thus emerge, in which cell topography would become more closely united with cell dynamics. It may help explain why genes, enzymes and viruses have a common type of structure, with nucleic acid united to proteins as a prosthetic group. It may also explain in part the role of the nucleic acids in neoplasia.

Again, in the distribution of nucleic acids in the cell, and with great clarity in the nucleus itself, we may be viewing the actual pathways by which the cell conducts its energy relations. The various forms which chromatin assumes, and the changes which it undergoes in the mitotic cycle, may really be a shifting series of pathways along which transfers of energy and substance are taking place between chromosomes and karyolymph and between nucleus and cytoplasm.

Such a concept would be of value in the pedagogy of cell structure and mitosis at all levels of instruction. The picture of the cell that we give to students in our elementary texts is unnecessarily static and lacking in orientation. Even in advanced courses in cytology and genetics, the student receives the impression of chromosomes floating about at random in the nucleus and mysteriously exerting their genetic influence across the nuclear space like so many suspended Svengalis. If the visible substance, nucleic acid, is involved in O/R-phosphorylation reactions, by which pH bond energy is generated along O/R pathways, then in its polarization in space, and its orderly behavior in time, we are looking at the cell in its actual functioning as an energy-transformer. Moreover, the whole cell should be so regarded and its morphology so taught. In developing his concept of "functional proteins", Green (57) points out that the various structures of the cell are also energy transformers; thus myosin converts chemical energy into mechanical energy, and visual purple converts light energy into chemical energy. Enzymes themselves, according to Green, "may ultimately turn out to be the energy transformers and converters of the cell".

As a corollary of the above, verification of the present hypothesis would call for a review and overhauling of the whole field of classical cytology. Many structures, configurations and sequences now without any apparent functional basis might be understood and much complicated and unconnected detail reduced to simpler terms and subsumed under a few simple principles which would better fit this discipline for the real task which lies ahead of it, namely, integration with the findings in the rapidly developing fields of enzyme chemistry and physiological genetics. Much the same service would be rendered to histology.

Pure descriptive cytology and histology are at present in a state of desuetude. I believe this is because of lack of simple principles governing the mass of topographic detail, and because cytology is organized as a handmaiden of genetics, and histology as an adjunct of pathology. If chromatin and chromosomes are shown to be lines of energy transfer and a part of the O/R phosphorylation mechanism for generating energy, then it will be seen that the accurate mapping of cytonuclear topography, not only in individual cells but in whole tissues, and not only in tissues but in successive stages of tissue development during embryogenesis and regeneration, is a task which remains to the pure descriptive microscopist. This information is vitally necessary to the development of physiological genetics, more specifically, to phenogenetics, and to a better knowledge and understanding of the forces and factors in operation during morphogenesis. Demonstration of the validity of this hypothesis, then, should stimulate the foundation and growth of a new branch of microscopical science, embracing both cytology and histology—a discipline which I believe is best designated by the term "cellular logistics". It is for this reason that I consider this and the preceding contribution as first papers in cellular logistics.

LITERATURE CITED

1. Avery, O. T. *et al.* Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Induction of transformation by a desoxyribonucleic acid fraction isolated from *Pneumococcus* Type III. Jour. Exp. Med. **79**: 137–157. 1944.
2. Balbiani, E. G. Sur la structure du noyau des cellules salivaires chez les larves de *Chironomus*. Zool. Anz. **4**: 637–641. 1881.
3. Bateman, J. B. Large molecules: their physical-chemical properties and their architectural and functional significance in living matter. *In* Höber's Physical chemistry of cells and tissues. Pp. 93–216. 1945.
4. Der Aufbau Chromosomen aus den Speicheldrüsen von *Chironomus Thummi.* Zeits. Zellf. Mikr. Abt. Allg. Zellf. u. Mikr. Anat. **23**: 280–313. 1935.
5. ————. Structure and arrangement of salivary gland chromosomes in *Drosophila* species. Proc. Nat. Acad. Sci. **22**: 216–222. 1936.
6. ————. Beiträge zur vergleichenden Morphologie der Speicheldrüsen Chromosomen. Zool. Jahrb. **56**: 239–276. 1936.
7. ———— *et al.* X-ray induced chromosomal alterations in *Drosophila melanogaster*. Genetics **23**: 610–630. 1938.
8. Beadle, G. W. Genes and the chemistry of the organism. Am. Scientist **34**: 31–53, 76. 1946.
9. Belar, K. Die cytologischen Grundlagen der Vererbung. Handb. Vererbungswiss. Vol. 1. 1928.
10. Berger, C. A. Multiple chromosome complexes of animals and polysomaty in plants. Cold Spring Harbor Symp. Quant. Biol. **9**: 19–22. 1941.
11. Biesele, J. J. *et al.* Nuclear phenomena in mouse cancers. Univ. Texas, Publ. 4243. 1942.
12. Bonnevie, K. Chromosomenstudien. I. Arch. Zell. **1**: 450–514. 1908.
13. Bowen, R. H. A preliminary report of the structural elements of the cytoplasm in plant cells. Biol. Bull. **53**: 179–196. 1927.
14. ————. Studies on the structuure of plant protoplasm. I. The osmiophilic platelets. Zeits. Zellf. **6**: 1. 1928.
15. ————. Studies on the structure of plant protoplasm. II. The plastidome and pseudochondriome. Zeits. Zellf. **9**: 1–65. 1929.
16. Brachet, J. Recherches sur la synthèse de l'acide thymonucléique pendant le developpement de l'oeuf d'oursin. Arch. Biol. **44**: 519–576. 1933.
17. Bridges, C. B. The structure of salivary chromosomes and the relation of the banding of the genes. Am. Nat. **69**: 59. 1934.
18. Briggs, R. Effects of growth inhibitor hexenolactone on frog embryos. Growth **10**: 45–73. 1946.

19. Buck, J. B. and R. D. Boche. Some properties of living chromosomes. Collecting Net 13: 201–203. 1938.
20. Callan, H. G. Heterochromatin in *Triton*. Proc. Royal Soc., London, B. 130: 324–335. 1941.
21. Carlson, C. G. The intergeneric homology of an atypical euchromosome in several closely related Acridinae. Jour. Morph. 59: 123–156. 1936.
22. Caspersson, T. and J. Schultz. Nucleic acid metabolism of the chromosomes in relation to gene reproduction. Nature 142: 294. 1938.
23. ————. Pentose nucleotides in the cytoplasm of growing tissues. Nature 143: 602–604. 1939.
24. ————. Ribonucleic acids in both nucleus and cytoplasm and the function of the nucleolus. Proc. Nat. Acad. Sci. 26: 507–515. 1940.
25. ———— *et al.* Zytoplasmatische Nucleotide in Tumorzellen. Naturwiss. 29: 29–30. 1941.
26. ———— and L. Santesson. Studies on protein metabolism in cells of epithelial tumors. 1942.
27. Child, C. M. Physiological foundations of behavior. 1924.
28. Claude, A. Distribution of nucleic acids in the cell and the morphological constitution of the cytoplasm. Biol. Symp. 10: 111–129. 1943.
29. Cohen, S. Streptomycin and desoxyribonuclease in the study of variations in the properties of a bacterial virus. Jour. Biol. Chem. 168: 511–526. 1947.
30. Cole, P. and E. Sutton. The absorption of ultraviolet radiation by bands of the salivary gland chromosomes of *Drosophila melanogaster*. Cold Spring Harbor Symp. Quant. Biol. 9: 66–71. 1941.
31. Coleman, L. C. The relation of chromocenters to the differential segments of *Rhoeo discolor*. Am. Jour. Bot. 28: 742–748. 1941.
32. Commoner, B. Structural relations in cell respiration. Quart. Rev. Biol. 17: 46–58. 1942.
33. Corey, I. Chromosome studies in *Stauroderus* (an Orthopteran). Jour. Morph. 55: 313–347. 1933.
34. ————. Heteropycnotic elements of Orthopteran chromosomes. Arch. des Biol. 49: 159–172. 1938.
35. Danielli, J. F. and D. G. Catcheside. Phosphatase on chromosomes. Nature 156: 294. 1945.
36. Darlington, C. D. Chromosome chemistry and gene action. Nature 149: 66–69. 1942.
37. ———— and L. La Cour. Nucleic acid starvation of chromosomes in *Trillium*. Jour. Genet. 40: 185–213. 1940.
38. ————. The detection of inert genes. Jour. Hered. 32: 115–121. 1941.
39. Darlington, C. D. and M. B. Upcott. The activity of inert chromosomes in *Zea mays*. Jour. Genet. 41: 275–296. 1941.
40. ———— and P. T. Thomas. Morbid mitoses and the activity of inert chromosomes in *Sorghum*. Proc. Royal Soc., London, B. 130: 127–150. 1941.
41. Demeric, M. The nature of changes in the white-notch region of the chromosome of *Drosophila melanogaster*. Proc. 7th Genet. Cong. 99–103. 1939.
42. ————. Genetic behavior of euchromatic segments inserted into heterochromatin. Genetics 25: 618–627. 1940.
43. Dobzhansky, T. Cytological map of the x chromosomes in *Drosophila melanogaster*. Biol. Zentr. 52: 493–509. 1933.
44. ————. Distribution of heterochromatin in the chromosomes of *Drosophila pallidipennis*. Am. Nat. 78: 193–213. 1944.

45. Doutreligne, J. Chromosomes et nucléoles dans les noyaux du type euchromocentrique. Cellule **42:** 31–100. 1933.
46. Dubos, R. J. The bacterial cell. 1946.
47. Emmens, C. W. The morphology of the nucleus in the salivary glands of four species of *Drosophila*. Zeit Zellf. Mikr. Anat. Abt. Allg. Zellf. Mikr. Anat. **26:** 1–20. 1937.
48. Engelhardt, W. A. *et al.* Chemistry and mechanics of the muscle studied on myosin threads. Compt. Rend. Acad. Sci. U. R. S. S. **30:** 644–646. 1941.
49. ————. Enzymatic and mechanical properties of muscle proteins. Yale Jour. Biol. Med. **15:** 21–38. 1942.
50. Fernandes, A. Sur le comportement d'un chromosome surnuméraire pendant la mitose. Sci. Gen. **1:** 141. 1939.
51. Fernandes, A. and J. A. Serra. Euchromatine et hétérochromatine dans leurs rapports avec le noyau et les nucléoles. Bol. Coc. Broteriana **19:** 67–124. 1944.
52. Fujü, S. An evidence for the presence of inert regions in the autosomes of *Drosophila virilis*. Cytologia **9:** 177–184. 1938.
53. Geitler, L. Das Wachstum des Zellkerns in tierischen und pflanzlichen Geweben. Ergebn. Biol. **18:** 1–54. 1941.
54. Goddard, D. Respiration of cells and tissues. *In* Höber's Physical chemistry of cells and tissues. 371–443. 1945.
55. Gordon, C. and J. H. Sang. Polygenic inheritance and the *Drosophila* culture. Nature **149:** 610–611. 1942.
56. Gowen, J. W. and E. H. Gay. Eversporting as a function of the Y chromosome in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **19:** 122–126. 1933.
57. Green, D. E. Enzymes and trace substances. Advances in Enzymology **1:** 177–198. 1941.
58. Greenblatt, R. B. *et al.* Experimental and clinical granuloma inguinale. Jour. Am. Med. Assoc. **113:** 1109–1115.
59. Gulick, A. The chemical formulation of gene structure and gene action. Advances in Enzymology **4:** 1–39. 1944.
60. Gutherz, S. Zur Kenntnis der Heterochromosomen. Arch. Mikr. Anat. **69:** 491–514. 1907.
61. Gullard, C. M. *et al.* The chemistry of the nucleic acids and proteins. Ann. Rev. Biochem. **14:** 175–206. 1945.
62. Heitz, E. Das Heterochromatin der Moose. Jahrb. Wiss. Bot. **69:** 762–818. 1928.
63. ————. Die Herkunft der Chromozentren. Planta **28:** 571–636. 1932.
64. ————. Die somatische Heteropyknose bei *Drosophila melanogaster* und ihre genetische Bedeutung. Zeits. Zellf. Mikr. Anat. Abt. Allg. Zellf. Mikr. Anat. **20:** 237–287. 1933.
65. ————. Über totale und partielle somatische Heteropyknose sowie Strukturelle Geschlechtschromosomen bei *Drosophila funebris*. Zeits. Zellf. Mikr. Anat. Abt. Allg. Zellf. Mikr. Anat. **19:** 237–287. 1933.
66. ————. Über A und B Heterochromatin sowie Konstanz und Bau der Chromomeren bei *Drosophila*. Biol. Zentr. **54:** 588–609. 1934.
67. ————. Chromostruktur und gene. Zeits. Ind. Abst. Ver. **70:** 402–447. 1935.
68. ————. Über mutative Intersexualität und Sexualität und Geschlechtsumwandlung bei den Lebermoosen *Pellia Neesiana* und *Sphaerocarpus Donnellii*. Naturwiss. **30:**. 751. 1942.
69. Helwig, E. R. The effects of x-ray upon the chromosomes of *Circotettix verruculatus*. Jour. Morph. **55:** 265–311. 1933.

70. ———. The frequency of reciprocal translocations in irradiated germ cells of *Circotettix verruculatus*. Arch. Biol. **49**: 143–158. 1938.
71. Hinton, Taylor. A comparative study of certain heterochromatic regions in the mitotic and salivary gland chromosomes of *Drosophila melanogaster*. Jour. Genet. **27**: 119–127. 1942.
72. Hughes-Schrader, S. The chromosome cycle of *Phenacoccus* (Coccidae). Biol. Bull. **69**: 462–468. 1935.
73. ———. A new type of spermiogenesis in iceryine coccids: with linear alignment of chromosomes in the sperm. Jour. Morph. **78**: 43–71. 1946.
74. Huie, L. Changes in cell-organs of *Drosera rotundifolia* produced by feeding with egg albumen. Quart. Jour. Micr. Sci. **39**: 387–426. 1897.
75. Janssen, L. W. Biosynthesis and the outlines of protein structures. Protoplasma **33**: 410–426. 1939.
76. Janssens, F. A. Spermatogenèse dans les Batraciens. III. Evolution des auxocytes mâles du *Batracoseps attenuatus*. Cellule **22**: 379–425. 1905.
77. Japha, Brigitte. Die Meiosis von *Oenothera*. II. Zeits. Bot. **34**: 321–369. 1939.
78. Kaufmann, B. P. Distribution of induced breaks along the X chromosome of *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **25**: 571–577. 1939.
79. Kodani, M. The structure of the salivary gland chromosomes of *Drosophila melanogaster*. I. The eu- and heterochromatin. Jour. Hered. **32**: 147–156. 1941.
80. Koller, P. C. Origin of malignant tumor cells. Nature **151**: 244–246. 1943.
81. Koltzoff, N. The structure of the chromosomes in the salivary glands of *Drosophila*. Science **30**: 312–313. 1934.
82. Kozhuchow, Z. A. Über die Natur der Extra-chromosomen bei *Zea mays*. Planta **19**: 91–116. 1933.
83. Krugelis, E. J. Distribution and properties of intracellular alkaline phosphatases. Biol. Bull. **90**: 220–233. 1946.
84. Lesley, M. N. The relation between satellite size and nucleolus size in three races of *Solanum lycopersicum*. Genetics **23**: 485–493. 1938.
85. Levan, A. Cytological reactions induced by inorganic salt solutions. Nature **156**: 751–752. 1945.
86. ———. Heterochromaticity in chromosomes during their contraction stage. Hereditas **32**: 449–468. 1946.
87. Lillie, F. R. Studies of fertilization in *Nereis*. Jour. Exp. Zool. **12**: 413–477. 1912.
88. Lillie, R. S. On the oxidative processes of the cell nucleus. Am. Jour. Physiol. **7**: 412–421. 1902.
89. Lippmann, Fritz. Metabolic generation and utilization of phosphate bond energy. Advances in Enzymology **1**: 99-158. 1941.
90. Longley, A. E. Supernumerary chromosomes in *Zea mays*. Agr. Res. **35**: 760–784. 1927.
91. Lorbeer, G. Die Umwandlung eines haploiden genotypisch weiblichen Gametophyten von *Sphaerocarpus Donnellii* in einen männlichen mit Hilfe von Röntgenstrahlen. Planta **25**: 70–83. 1936.
92. Mampell, K. Genic and non-genic transmission of mutator activity. Genetics **31**: 496–505. 1946.
93. Mather, K. Crossing over and heterochromatin in the X chromosome of *Drosophila melanogaster*. Genetics **24**: 413–435. 1939.

94. ————. Variation and selection of polygenic characters. Jour. Genet. **41**: 159–192. 1941.

95. ————. Polygenic balance in the canalization of development. Nature **151**: 68–71. 1943.

96. ————. The genetical activity of heterochromatin. Proc. Royal Soc., London, B **132**: 308–332. 1944.

97. McClintock, B. The relation of a particular chromosomal element to the development of the nucleoli in *Zea mays*. Zeits. Zellf. Mikr. Anat. Abt. Allg. Zellf. Mikr. Anat. **21**: 294–328. 1934.

98. ————. *Neurospora*. I. Preliminary observations of the chromosomes of *Neurospora crassa*. Am. Jour. Bot. **32**: 671–678. 1945.

99. Metz, C. W. and M. L. Schmuck. Differences between chromosome groups of soma germline in *Sciara*. Proc. Nat. Acad. Sci. **17**: 272–275. 1931.

100. ———— and E. H. Gay. Chromosome structure in the salivary glands of *Sciara*. Science **80**: 295–296. 1934.

101. ————. Structure of salivary gland chromosomes. Cold Spring Harbor Symp. Quant. Biol. **9**: 23–39. 1941.

102. Mitchell, J. J. Disturbances of nucleic acid metabolism produced by therapeutic doses of X and gamma radiations. III. Inhibitions of synthesis of thymonucleic acid by radiation. Brit. Jour. Exp. Path. **23**: 309–313. 1942.

103. Mitchell, P. H. A text book of biochemistry. 1946.

104. Muller, H. J. and A. A. Prokofyeva. The structure of the chromosomes in the inert region of the X chromosome of *Drosophila*. Compt. Rend. Acad. Sci. U. R. S. S. **1**: 658–660. 1935.

105. ———— and S. M. Gershenson. Inert regions of chromosomes as the temporary products of individual genes. Proc. Nat. Acad. Sci. **21**: 69–75. 1936.

106. ———— *et al.* A further analysis of loci in the so-called "inert region" of the X chromosome of *Drosophila*. Genetics **22**: 87–93. 1937.

107. ————. The remaking of chromosomes. Collecting Net **13**: no. 8. 1938.

108. ————. Induced mutations in *Drosophila*. Cold Spring Harbor Symp. Quant. Biol. **9**: 151–167. 1941.

109. ————. Résumé and perspectives of the symposium on genes and chromosomes. Cold Spring Harbor Symp. Quant. Biol. **9**: 290–308. 1941.

110. ———— and T. S. Painter. The differentiation of the sex chromosomes of *Drosophila* into genetically active and inert regions. Zeits. Ind. Abst. Vererb. **62**: 316–365. 1932.

111. ————. The position effects as evidence of the localization of the immediate products of gene activity. Summ. Commun. XV Int. Physiol. Congress, Leningrad-Moscow. 587–589. 1935.

112. Neuhaus, M. Additional data on crossing over between X and Y chromosomes in *Drosophila melanogaster*. Genetics **22**: 333–339.

113. Noujdin, N. J. Role of structural homo- and hetero-zygosity in mosaic formation. Nature **155**: 514. 1945.

114. Painter, T. S. and A. N. Taylor. Nucleic acid storage in the toad's egg. Proc. Nat. Acad. Sci. **28**: 311–317. 1942.

115. ————. Cell growth and nucleic acids in the pollen of *Rhoeo discolor*. Bot. Gaz. **105**: 58–68. 1943.

116. ————. Nuclear phenomena associated with secretion in certain gland cells with especial reference to the origin of cytoplasmic nucleic acid. Jour. Exp. Zool. **100**: 523–541. 1945.

117. ———— and A. B. Griffen. The structure and the development of the salivary gland chromosomes of *Simulium*. Genetics **22**: 612–633. 1937.

118. Pavan, C. Two types of heterochromatin in *Drosophila nebulosa.* Proc. Nat. Acad. Sci. **32**: 137–145. 1946.
119. Pollister, A. W. Centrioles and chromosomes in the atypical spermatogenesis of *Vivipara.* Proc. Nat. Acad. Sci. **25**: 189–195. 1939.
120. Pontecorvo, G. Structure of heterochromatin. Nature **153**: 365–367. 1944.
121. Potter, V. R. Biological energy transformation and the cancer problem. Advances in Enzymology **4**: 201–256. 1944.
122. Poulson, D. F. and C. W. Metz. Studies on the structure of the nucleolus forming regions and related structures in the giant salivary gland chromosomes of Diptera. Jour. Morph. **63**: 363–395. 1938.
123. Prokofyeva, A. A. Morphologische Struktur der Chromosomen von *Drosophila melanogaster.* Compt. Rend. Acad. Sci. U. R. S. S. **4**: 102–107. 1934.
124. Prokofyeva-Belgovskaya, A. A. Y chromosome in salivary glands of *Drosophila.* Compt. Rend. Acad. Sci. U. R. S. S. **3**: 365–366. 1935.
125. ———. The structure of the Y chromosome in the salivary glands of *Drosophila melanogaster.* Jour. Genet. **22**: 94–103. 1937.
126. ———. Cytological mechanism of mosaicism and of chromosome rearrangement. Compt. Rend. Acad. Sci. U. R. S. S. **22**: 270–273. 1939.
127. ———. Struktura khromotsentra. Compt. Rend. Acad. Sci. U. R. S. S. 498–499. 1935.
128. Resende, F. Heterochromatine. Portugaliae-Acta Biol. **1**: 139–156. 1945.
129. Ribbands, C. R. Meiosis in Diptera. Prophase associations of non-homologous chromosomes and their relation to mutual attraction between centromeres, centrosomes and chromosome ends. Jour. Genet. **41**: 411–442. 1941.
130. Richards, A. History of the chromosomal vesicles in *Fundulus.* Biol. Bull. **32**: 249–282. 1917.
131. Schrader, F. Experimental and cytological investigations of the life cycle of *Gossyparia spuria* (Coccidae) and their bearing on the problem of haploidy in males. Zeits. Wiss. Zool. **134**: 149–179. 1929.
132. Schultz, J. Variegation in *Drosophila* and the inert chromosome regions. Proc. Nat. Acad. Sci. **22**: 27–33. 1936.
133. ——— and D. G. Catcheside. The nature of X chromosomes in *Drosophila melanogaster.* Jour. Genet. **35**: 315–320. 1937.
134. ———. The function of heterochromatin. Proc. 7th Int. Genet. Congress. 257–262. 1939.
135. ———. The evidence of the nucleo-protein nature of the gene. Cold Spring Harbor Symp. Biol. **9**: 55–65. 1941.
136. ———. Physiological aspects of genetics. Ann. Rev. Physiol. **5**: 35–62. 1945.
137. Scott, C. H. The deposition of the fixed mineral salts during mitosis. Bull. Histol. Appliq. **7**: 251–256. 1930.
138. Soskin, S. and R. Levin. Carbohydrate metabolism. 1946.
139. Stadler, L. J. On the genetic nature of induced mutations in plants. Proc. 6th Nat. Congress Genetics. 274–294. 1932.
140. Stern, C. Ein genetischen und zytologisches Beweis für Vererbung in Y Chromosomen von *Drosophila melanogaster.* Zeits. Ind. Abst. Vererb. 1927.
141. Stowell, R. E. Thymonucleic acid in tumors. Cancer Res. **5**: 283–294. 1945.
142. Sutton, E. The structure of the salivary gland chromosomes of *Drosophila melanogaster* in exchanges between euchromatin and heterochromatin. Genetics **25**: 534–540. 1940.

143. Szent Gyorgi, A. Towards a new biochemistry. Science **93**: 609–611. 1941.

144. ————. Contraction and the chemical structure of muscle fibril. Jour. Colloid. Sci. **1**: 1–19. 1946.

145. Van Beneden, P. Nouvelles recherches sur la fécondation et la division mitosique chez l'*Ascaris megalocephala*. 1887.

145a. Vanderlyn, Leon. Somatic mitosis in the root tip of *Allium Cepa*—A review and a reorientation. Bot. Rev. **14**: 270–318. 1948.

146. Wenrich, D. H. The spermatogenesis of *Phrynotettix magnus* with special reference to synapsis and the individuality of the chromosomes. Contr. Zool. Lab. Mus. Comp. Zool., Harvard Coll. No. 266: 57–133. 1916.

147. Westergaard, M. Structural changes of the Y chromosome of polyploid *Melandrium*. Hereditas **32**: 60–64. 1946.

148. Wharton, L. T. Analysis of the metaphase and salivary chromosomes morphology within the same genus. *Drosophila*. Univ. Texas, Publ. 4313: 282–303. 1943.

149. White, M. J. D. The heteropycnosis of sex chromosomes and its interpretation in terms of spiral structure. Jour. Genet. **40**: 67–82. 1940.

150. ————. Amount of heterochromatin as a specific character. Nature **152**: 536–537. 1943.

SEVENTH INTERNATIONAL BOTANICAL CONGRESS, STOCKHOLM, 1950

The Seventh International Botanical Congress will be held in Stockholm between July 12 and 20, 1950. Presessional excursions will start on June 27 and postsessional will end on August 9. Communication No. 2 from the Organizing Committee containing the preliminary outline of the program during the meetings and the preliminary plans for the excursions can be obtained from the Secretary General Dr. Ewert Åberg, Uppsala 7, Sweden.

Outstanding McGRAW-HILL Books

PLANT AND SOIL WATER RELATIONSHIPS

By Paul J. Kramer, Duke University. *McGraw-Hill Publications in the Botanical Sciences.* 340 pages, $4.50

Gives an integrated discussion of the various factors which affect the absorption of water by plants. These factors include the availability of soil moisture, the development of efficient root systems, the nature of the absorption process, the relation between the absorption of water and the absorption of minerals, and the relation between water absorption and other plant processes. The fundamental scientific principles are explained as simply as possible, and many applications of these principles are described.

BOTANY. A Textbook for Colleges

By J. Ben Hill, Lee O. Overholts, and Henry W. Popp, The Pennsylvania State College. *McGraw-Hill Publications in the Botanical Sciences.* Second edition. In press

This book has been revised carefully in order to record advances in the field in recent years and to eliminate shortcomings revealed by teaching of the original edition. This revised edition treats recent advances in the science in photosynthesis, respiration, fermentation and enzymes, metabolism, hormones and vitamins, antibiotics, the use of ''tagged elements,'' hydroponics, etc. New sections have been added on viruses, actinomycetes, diatoms, the economic importance of fungi as well as a special section on meiosis which is probably unique in general textbooks of botany.

FIELDBOOK OF NATURAL HISTORY

By E. Laurence Palmer, Cornell University. 682 pages, textbook edition, $5.00

Here, in one convenient volume, is an extraordinarily comprehensive guide to natural history. The book includes birds, fish, plants, rocks and minerals, the stars, mollusks, and mammals—more than 2,000 items and hundreds of pictures arranged and tabulated for easy reference.

FIELD MANUAL OF PLANT ECOLOGY

By Frank C. Gates, Kansas State College and University of Michigan Biological Station. *McGraw-Hill Publications in the Botanical Sciences.* 142 pages, $3.00

This manual gives directions for exercises and experiments which will enable the student to begin a study of plant ecology in the field, to gather data which can be used to describe various types of vegetation and also to study individual plants in relation to their environment.

Send for copies on approval

McGRAW-HILL BOOK COMPANY, INC.
330 WEST 42ND STREET, NEW YORK 18, N. Y.

THE BOTANICAL REVIEW

VOL. XV NOVEMBER, 1949 No. 9

THE DEVELOPMENT OF ROOT HAIRS IN ANGIOSPERMS

R. G. H. CORMACK[1]

University of Alberta

INTRODUCTION

Studies in developmental morphology show a growing tendency to emphasize causal factors. There is also an increasing tendency on the part of investigators to apply the experimental method to studies in this field. In short, the essence of this approach is the subjection of a well defined plant structure to an intensive developmental study and the verification and modification of the hypotheses which invariably arise. Sifton (84), in pointing out the merits of the experimental method, states that in this way "The investigator not only gains insight into the particular, perhaps very limited and specialized, subject of his investigation, but in doing so uncovers points of general application that have, from lack of such work, hitherto been overlooked".

What is true of studies of developmental morphology in general, is also true of studies relating to the development of root hairs. Almost from the beginning investigators in this field have been assailed by questions of cause; for instance: why does a protuberance arise and why does it grow into a cylindrical hair; why does one epidermal cell form a hair and a neighboring cell remain hairless; and why do the roots of some plants form hairs under certain conditions and fail to do so under others? These questions have not always been attacked specifically, and the data in many cases are widely scattered. Snow's paper (88) includes a satisfactory review of the work done on root hairs prior to 1905. Since then Farr

---

[1] The author wishes to thank Prof. H. B. Sifton, Dept. of Botany, University of Toronto, for his kindness during the early years of this study, and Dr. M. J. Huston, Director, School of Pharmacy, University of Alberta, for his interest and kindness in reading the manuscript.

(33) has summarized the literature on root-hair elongation, while Miller (65) has dealt in detail with the physiology. Thus the object of the present paper is to discuss the problem from the developmental standpoint. Such a treatment will place the emphasis directly on the hair-forming cells and on the various factors which influence their growth and behaviour.

THE EPIDERMIS

The epidermal cells of roots, whether they produce root hairs or not, are the most important absorbing cells in higher plants. Besides their connection with the phenomena of absorption, their superficial position and arrangement in longitudinal rows lend themselves readily to microscopic observation.

In view of these facts it is not surprising that root epidermal cells have received a great deal of attention. The three points to be considered under this heading in the present review are the condition of the epidermis, initiation of the root hairs and succession of them.

Condition of the Epidermis. As seen in surface view, the root epidermis consists of long vertical rows of elongate thin-walled parenchyma cells which fit closely together with no intercellular spaces between them. Contrary to general opinion, the epidermis is not the same for all roots. In some roots it is clearly differentiated into short and long cells, while in others, though all the cells may appear to be of the same length, they are found on close examination to vary greatly in length in the same region and from one region to another in the same root. Consequently numerous attempts have been made to establish a relationship between cell-length and root-hair production.

Several investigators have associated specialized epidermal cells with root hairs. Where this condition is most obvious, as in the roots of certain water plants (94, 81, 53), the hair-forming cells are much shorter than the rest, being cut off by transverse walls at an early stage of development. Not only are they shorter than the adjacent hairless cells but they differ often in shape and in cell contents. Leavitt (56) made a critical study of this specialized type of hair-forming cell and called them "trichoblasts". He found them not only in water-plant roots but also among the higher cryptogams and certain monocotyledons. More recently Wilson (99) has observed them in the roots of *Elodea canadensis, Potamogeton densus* and *Alisma plantago.* In these roots the trichoblasts are identifiable in the meristematic region long before the hairs are developed. At this stage they have thicker walls, larger nuclei and denser protoplasm than the long hairless type of cell. Because of certain peculiarities in the development of the hairs, *Elodea* roots have been the subject of considerable experimentation (13, 50), the results of which will be discussed later under another heading.

Some work has been done on roots that lack the well-defined trichoblastic type of cell. In this type of root, all the cells appear alike, and any cell seems capable of forming a hair. From her measurements of the epidermal cells of this type of root, namely, corn, wheat, peas, beans and squash, Snow (88) concludes that, although no definite length can be given as the limit for hair development, the average length of the hair-forming cells is less than that of the hairless cells. In her opinion it is the differential elongation of the epidermal and cortical cells that is significant and hair-production in roots of this kind depends on the ratio between the capacity of the epidermal cells to elongate and their ability to do so. Bardell (3) reaches the same conclusion, but Roberts (78) does not agree with this view and does not approve of the experimental data. The latter's measurements for roots of this type show that for corresponding epidermal and cortical cells there is no definite relation between cell length and hair-forming potential. In the opinion of the present writer, none of these workers, with the exception of Roberts, considered the extreme variability in epidermal cell-length that may occur in roots of this type. Consequently their measurements, taken at random over the whole root surface, are of little value in trying to establish a relationship between cell-length and root-hair production.

Recent attempts to establish a relationship between epidermal cell-length and root-hair development are the most pertinent. In his studies concerning the development of root hairs, the present writer (12–17) distinguishes two main types of root epidermis, namely, differentiated and undifferentiated. For instance (12), in cabbage and white mustard roots the epidermis is differentiated into short and long cells. However, in these roots the short cells are arranged in distinct rows and are much less specialized than the trichoblasts of Leavitt. Normally the short cells produce the hairs, while the long cells remain hairless. Under ordinary conditions root hairs are abundantly developed, but they are not always uniformly spaced. Alternating zones of longer and shorter cells occur, either normally or as a result of changing the environmental conditions. In the most sparsely haired zone, epidermal cell-length is at a maximum and some or all of the short cells may be hairless, while in the most densely haired zones, epidermal cell-length is at a minimum and even the long cells form hairs. In the other type of epidermis (12, 15), exemplified by roots of tomato, corn and endive, any cell seems capable of forming a hair, and both hairless and hair-producing cells appear to be much the same in length. However, when measurements are restricted to short distances or to well marked zones of longer and shorter cells, a definite relationship between epidermal cell-length and root-hair development is clearly evident. Not only is this true, but there is also a well marked relationship between epidermal cell-length and length of the full grown hair. The longest cells either are hairless or merely produce papillae, while the shortest cells produce the longest hairs. In both types of root epidermis, however, the formation of root hairs is markedly dependent on external conditions which modify the hardening of the epidermal cell walls. As will be described later, this hardening comes about through gradual incorporation of calcium into the outer wall layer of the growing hair.

Two main types of root epidermis are recognized by Sinnott and Bloch (85–87, 4) among certain related genera of the grass family. In one type, exemplified by *Phleum* and *Poa,* the last division in the meristematic region is an unequal one which results in a small trichoblast and a larger cell which does not form a hair. The trichoblast, which is more richly protoplasmic, is always toward the tip of the root, and the hairless cell toward the base. During elongation the trichoblasts grow less than the hairless cells and at maturity form the hairs. In roots of the other type, namely, *Sporobolus* and *Chloris,* the last division is more equal, and both daughter cells may form a hair. The occurrence of trichoblasts in *Phleum* roots has been subsequently confirmed by Brumfield (8) and Cormack (14), but the latter has observed that considerable variation in the length of both trichoblastic and hairless cells may occur and that under suitable conditions both types of cells will form hairs.

Root-Hair Initiation. The emergence of a hair and its position in respect to the epidermal cell from which it originates present interesting problems which have received relatively little attention in relation to their importance. This may explain in part the confusion which exists in text-books in this connection. In certain cases the text-book figures are inaccurate; in others they are atypical with no reference to the identity of the plant; and in still others it is difficult to ascertain whether the hair is just beginning to grow or has reached its final size.

According to Snow (88), in corn roots the hair originates near the tip of the root where the cells are isodiametric. Nearly the whole wall curves at first, but as the epidermal cell continues to elongate, this primary swelling becomes a papilla. Subsequent study, however, has shown that corn roots are extremely sensitive to changes in the environmental conditions and that considerable variation occurs in the formation of the hairs. Roberts (78), working with endive and lettuce roots, observed that the initial formation of the root hair is indicated by a general swelling, and that further swelling, followed by growth, takes place at the less resistant portion of the wall. Robert's concept of root-hair emergence has been further developed by the present writer (12) in his study of root-hair development in cabbage and white mustard roots. In these roots the hair emerges as a small bulge invariably near the apical end of the epidermal cell. There is strong evidence that the wall is softer at this point and that longitudinal growth of the epidermal cell has ceased in these roots by the time a hair begins to push out. Under favourable conditions, where the epidermal cell walls harden gradually but rapidly, the hair emerges at the extreme end of the cell, often projecting over the cell immediately below.

In their study of cell polarity and the differentiation of root hairs in related genera of small-seeded grasses, Sinnott and Bloch (86) have shown that there are markedly different types of root-hair determination. For example, in *Phleum* the hair originates from near the apical end of the trichoblast, while in *Sporobolus* it appears to originate near the middle of the cell. From these observations they emphasize the importance of certain inherent qualities of the protoplasm in the development of root hairs and assume that there is a strong apical tendency in *Phleum* roots and a much weaker tendency in *Sporobolus*. This hypothesis is certain to meet with criticism if for no other reason than that it does not interpret the nature of the mechanism. It is very apparent that biological phenomena are inextricably involved with the function of the living protoplasm. However, to say that the protoplasm influences root-hair determination without explaining how and to what extent seems to the present writer to be merely evading the issue.

After re-investigating the development of root hairs in these two grasses, the present writer (14) has arrived at a practical interpretation that takes into consideration the influence of the environmental conditions in root-hair determination. Critical study showed that the original position of the emerging hair is apical in both roots but that its final position is dependent on the capacity of the hair-forming cell to grow longitudinally after a hair has begun to push out. For instance, in *Phleum* the hair originates as a narrow bulge at the apical end of the hair-forming cell when that cell has reached or almost reached its final length. Consequently in this root the apical position of the hair is maintained. On the other hand, in *Sporobolus* the hair originates as a wide bulge or papilla at or very near the apical end of a young cell, while in a mature cell its position is usually more central. This evidence indicates that the epidermal cells are still growing in length in the hair-producing region of *Sporobolus* roots. This view is further supported by work (15) with tomato roots, the epidermis of which is similar to that of *Sporobolus.* In this study careful measurements show that the epidermal cells are still growing in the region where the papillae first make their appearance and that the apical end of the cell grows more rapidly than the basal end. Accordingly the final position of the mature papilla, or hair, will depend on the length of time the apical end continues to grow. If this period is short the original apical position will be more or less maintained; if greatly protracted, the papilla will be found in the middle and occasionally in the basal portion of the full-grown cell. As in previous studies with roots of other plants, the formation of root hairs is shown to be dependent on external conditions whose effect is produced by their ability to modify the hardening of the epidermal cell walls.

In connection with the emergence of root hairs, the position of the nucleus has not been overlooked. Haberlandt (39) observed that the nucleus lies along the exterior wall in the vicinity of the incipient protuberance, and when further growth takes place the nucleus follows along at a rather uniform distance behind the growing root-hair tip. Windel (100) and Farr (24) have corroborated Haberlandt's observations, though in a later study with bifurcated hairs the latter (32) believes that the nucleus may sometimes have a retarding effect on root-hair growth by interferring with protoplasmic streaming. Previous to this, Küster (54) and Roberts (78) were not convinced of any relation between the position of the nucleus and the original swelling of the epidermal cell wall. The most recent observations (15, 86) support those of Haberlandt, but how the nucleus influences the initiation of a root hair is still a matter of conjecture.

Succession of Root Hairs. It is common knowledge that root hairs develop acropetaly, the first hairs appearing very close to the root apex. The acropetal development of root hairs was first observed by Schwarz (82) and, though denied by Devaux (19), has been generally accepted. Haberlandt (40) in particular emphasizes the fact that new hairs never develop among existing ones.

In most seedling roots the acropetal development of root hairs results in a uniform gradation in the length of the hairs beginning with those nearest the root apex. In some roots, however, a mingling of long and short hairs is observed. Roberts (78) has noticed the mingling of papillae and hairs when roots of alfalfa, cabbage and *Verbascum* are grown in moist air, but no definite conclusions of its cause are reached. Pinkerton (69) has described the mingling of long and short hairs in the roots of lettuce seedlings and concludes that in this case new hairs could arise at various indiscriminate places in the piliferous layer. Once again, however, it was not made clear whether the shorter hairs were actually new ones or whether they had failed to elongate to the same extent as the longer hairs. The present writer's observation (15) that in tomato roots the epidermal cells continue to elongate in the hair-producing region may explain the mingling of hairs of different lengths. Careful measurements showed that the length of time that elongation continues at the apical end of the hair-forming cell determines the final position of the papilla in the mature cell and also determines whether a papilla remains or grows into a hair. Thus where papillae are mixed with short hairs in the mature region of this root they are not new, but represent papillae whose development is arrested almost as soon as they begin to push out.

Conclusive proof of acropetal development of root hairs is demonstrated in certain cultural experiments carried out with cabbage roots (12). When roots growing in a solution that represses root-hair development are transferred to a solution that stimulates development, marked recovery, characterized by a sudden burst of long straight hairs, always results. The first cells to produce hairs in the zone of new growth are those that are still elongating when the roots are transferred to the favourable solution. Those cells which have completed elongation never push out hairs, and consequently the region above the zone of new growth remains unchanged. Thus there seems little doubt that once a certain stage in their development has been reached, epidermal cells no longer have the capacity to form hairs.

The only authentic record of new hairs developing among preexisting hairs is that reported by Pinkerton (69) for the roots of certain members of the Commelinaceae. Here, however, the new hairs are most certainly of cortical origin, superseding the primary epidermal hairs, and their absorptive ability is doubtful.

In general, root hairs are short lived, collapsing and withering away at the end of a few days or weeks. Following collapse of the hairs the walls of the epidermal cells, if not completely worn away, become suberized or lignified. However, certain exceptions have been recorded. In *Gleditsia triacanthos* (59) the hairs become very thick walled and may persist for several months. Persistent root hairs have been observed in certain species of the Compositae (98) and in a number of other plants (21). However, in these roots it seems unlikely that the persistent root hairs continue to absorb water for that length of time.

Thus, according to the literature just reviewed, the condition of the epidermis is not the same for all roots. In some roots there is a very strong morphological differentiation between the hair-forming cells (trichoblasts) and other epidermal cells, in others a smaller difference, and in still others none at all. In all cases there is a definite relationship, however, between epidermal cell-length and root-hair production and also between cell-length and length of the full-grown hairs, the relationship being that the longest epidermal cells either are hairless or produce the shortest hairs, while the shortest epidermal cells produce the longest hairs. This relationship strongly suggests that each epidermal cell has a certain capacity for growth which may be expressed in either a longitudinal or horizontal direction. In every case where it has been critically studied we find that the hair emerges at or near the apical end of the hair-forming cell. However, its position at maturity depends on whether the hair-forming cell has stopped growing longitudinally at the time the hair begins to push out. If it has, the extreme apical position of the hair will be maintained; if not, the papilla or hair will assume a more central position. It is now established that root hairs develop acropetally and that new hairs never originate among pre-existing hairs.

CHEMICAL COMPOSITION OF ROOT-HAIR CELL WALLS

Critical studies of root-hair cell walls, as related to the growth of those structures, had their beginning with the experiments of Stiehr in 1903 (91). He showed that stretching of the cell wall takes place more readily at the tip of the hair than along the sides, and that when bursting occurs, the rupture is invariably at this point. From these observations he has taken the view that the end wall of the hair is of softer materials. Experiments to test the validity of this view have repeatedly strengthened it, despite wide disagreement as to the exact difference between the hypothetical soft area at the tip of the growing hair and the rest of the cell wall. There is considerable controversy in the earlier work as to the presence of cellulose. A number of non-cellulosic substances were identified whose presence in the root hair wall might account for greater plasticity during growth of the hair.

Of the earlier workers, Ridgway (77) failed to obtain a positive test for cellulose but was the first to observe the occurrence of a non-cellulosic material called "callose" in the cell walls of certain root hairs. According to Miller (65), the chemical nature of callose is not known, but it differs from other cell wall materials in its solubility and staining reactions. Its presence in the root-hair cell wall would imply greater plasticity than if the wall were to consist of cellulose alone. Roberts (78), working with the root hairs of a number of common crop plants grown in moist air, seems to have been the first to note that the root-hair cell wall is continuous with the walls of the hair-forming cell, and that it is made up of two parts, an inner layer of cellulose and an outer layer of calcium pectate. The cellulose layer, although thin, is uniform over the hair, while the pectic layer appears somewhat thinner at the tip where it is sometimes associated with a special covering of callose. Howe (47), working with the root hairs of an even larger number of common plants, grown mainly in loam and sand, also found that the wall of the hair-forming cell consists of an inner layer of cellulose and an outer layer of pectic material. However, in her opinion the outer pectic layer of this wall is continuous over the hair, while the inner cellulose layer is not. In the hair itself the inner wall layer is of callose. To explain this difference in composition of the inner layer she suggests that as the epidermal cell bulges to form a hair, the cellulose inner lamella stretches, then breaks, and no more cellulose is formed. However, this unusual mechanical interpretation has never been confirmed and is no longer tenable in the light of more recent investigations. We know now that cellulose is a constituent of the epidermal cell from an early stage in its development and that, when a hair pushes out, the cellulose layer extends outwards unbroken over the whole inner surface of the root-hair wall.

Ziegenspek (103) identified cellulose along the side walls of the hair but not at the tip. Here he identified another cell wall material called "amyloid". This substance is distinguished from cellulose in that it gives the blue colour test directly with iodine without further treatment with sulphuric acid. He considers the plastic amyloid to be a transitional product in the formation of cellulose, and he believes its presence at the tip makes possible the growth of the hair. Since then some doubt has been cast on the specificity of the amyloid reaction. Meeuse (61, 62) believes that it is merely indicative of a low quantity of cellulose in the walls of young growing tissues, and cites evidence obtained by other investigators to the effect that cellulose is formed immediately by the protoplasm from glucose. The occurrence of amyloid in root hairs has never been confirmed (46, 58, 12), and a theory of root-hair growth has been elaborated independent of the amyloid stage (12). No further microchemical treatment of the problem appeared until Addoms (1) recorded her failure in 1927 to obtain a satisfactory test for either cellulose or pectic materials, thus showing that more critical study was needed.

That some of the confusion arising out of the foregoing work was due in part to the use of diverse plant material and in part to failure to distinguish between actively growing hairs and fully grown hairs, is no doubt true. However, that it was mainly due to unreliable methods of testing for cell wall materials is indicated by the almost complete agreement among recent workers, following the introduction of new methods and improvements in microchemical techniques. Modern researches, if they have done nothing else, have served to bring our knowledge of the root hair into line with that of other growing cells of the parenchymatous type whose thin primary wall consists of cellulose and pectic materials.

Hopmann (46) casts doubt on Zeigenspek's theory of root-hair growth. He reports the absence of amyloid from root hairs developed in water and the presence of cellulose over the whole inner surface under all condition.

McCoy (58), in search of an explanation for the entry of nodule-forming bacteria into the root hairs of pea, lucerne, broadbeans and clover roots, has made a thorough study of the root-hair wall. Differential solubility and staining reactions leave no doubt as to the presence of cellulose in the root hair wall, even to the tips of both young and old hairs. However, due to the presence of a very resistant hemicellulose, especially at the tips, fresh tissue does not give the characteristic blue colour with chlor-zinc-iodine nor with other cellulose test reagents without previous treatment with dilute alkali or eau de javelle. This may explain the failure of some of the earlier workers to obtain a positive cellulose reaction. Calcium pectate occurs, though its behaviour with certain dissolving reagents suggests that it exists in association with cellulose and not as a distinct outer layer. Special attention was given to the point of entry of the nodule-forming bacteria. Invariably they attack a hair at the tip, and, since no chemical difference could be found at this point, a physical difference in the wall at the tip is suggested to account for this phenomenon. That there is some difference here is clearly shown by a simple experiment where the roots of lucerne and clover seedlings, grown on the surface of mineral-salts agar, are watered with sterile distilled water. Within a short time the root hairs begin to swell into queer club-shaped and bulbous forms, the tips being first affected.

The results of a similar study by the present writer (12) with the roots of cabbage seedlings are in close agreement with those of

McCoy and leave no doubt as to the presence of an inner layer of cellulose and an outer layer of calcium pectate. In this study, slight, but none the less important, differences are noticeable in the walls of young actively growing hairs and in those of mature fully-grown hairs. In the walls of the former a thin layer of cellulose, continuous with the wall of the epidermal cell, extends along the sides of the hair and over the tip. During growth the cellulose layer is soft and easily hydrolyzed by acid. The pectic layer along the sides of the growing hair is of firm calcium pectate. On the dome-shaped tip, this layer is composed either of pectic acid or of a softer kind of calcium pectate. In either case the wall covering the tip is softer and more delicate than that along the sides.

Proof of the more yielding nature of the tips of growing hairs is demonstrated when roots grown in a concentrated calcium salt solution are transferred to distilled water on a slide. Under these conditions the hairs burst, and the bursting, as in the hairs with which Stiehr, Roberts and McCoy worked, takes place always at the tips. The results of microchemical tests, and evidence obtained from experiments with pectic acid *in vitro,* point to the conclusion that this increased plasticity at the tip is due to a difference in the pectic layer and not to any difference in the cellulose. Further evidence in support of this conclusion is supplied by the results of numerous cultural experiments. Where calcium is readily available, the softer more plastic area is confined to a narrow area at the tip, with the result that the growing hairs become long and straight and exceedingly narrow. On the other hand, where calcium is not readily available, the softer area becomes greatly extended with the result that the growing hairs become inflated, bulbous at the base and variously branched. Thus the normal hair is always such as can be explained on the basis of internal pressure on a wall that has been gradually and equally hardened, while the abnormalities are always such as can be explained on the basis of internal pressure on a wall that has been unequally hardened.

In the mature full-grown hair the cellulose layer is thicker and less easily hydrolyzed by acid, and the whole surface of the hair including the tip is covered by a layer of calcium pectate of the firmer type. The possible presence of a pectocellulose complex such as protopectin (92, 5) in these hairs is suggested by the greater difficulty of dissolving out either cellulose or calcium pectate.

Certain cell wall differences were found to explain differences in behaviour between the hair-forming short cells and the hairless long cells of cabbage roots. Under ordinary conditions in the region where hairs are initiated, the pectic layer of the short cells consists of calcium pectate, while that of the long cell walls is of pectic acid. The long cells are found to be considerably more acid than the short cells at this stage. At a somewhat later stage they become less acid, and the pectic acid layer of their walls changes to calcium pectate. Experiments proved that calcium fails to unite with pectic acid in an acid medium, thus providing the clue to this behaviour. Under conditions where the change to calcium pectate is speeded up the long cells produce normal hairs.

Root hairs with lignified cell walls have been reported in a number of special cases (59, 43), while hairs with heavily cutinized walls have been observed among certain of the Commelinaceae (69). The latter, however, are of cortical origin, and it doubtful that they have the capacity of absorption. Further microchemical studies of these hairs would be interesting in the light of more recent work on the development of such structures.

In summing up, the root-hair wall consists of cellulose and calcium pectate continuous with corresponding layers in the hair-forming cell. During growth the wall of the root-hair tip remains active and more yielding than that of the sides, and present evidence indicates that differences in plasticity are brought about by an increasing degree of calcification of the pectic layer from the tip of the hair to the base. In a recent review Farr (37) emphasizes the importance of non-cellulosic materials in enlarging cell walls. Using root hairs as an example she draws the following conclusion: "Root hairs represent a special group of cells in which wall plasticity is of continued importance, and cellulose formation and deposition rarely take place". While it is true that the physical properties of the root-hair wall are largely determined by the nature of the pectic materials, yet recent studies (46, 58, 12) show conclusively that cellulose is present in the root-hair wall at every stage of its development. Despite divergent opinions as to the way in which the wall of the hair at the tip differs from that of the rest of the hair, the apical growth of root hairs seems definitely established (101, 102, 91, 83, 33, 76, 40).

FACTORS AFFECTING ROOT-HAIR DEVELOPMENT

Moisture. The effect of moisture on root-hair development has been extensively but inconclusively studied by growing roots in soil and air with different percentages of moisture, and in water. According to the evidence reviewed by Schwarz (82), the earliest workers reported a close relationship between moisture and root-hair production. In his own experiments with corn and wheat roots, Schwarz concludes that there is a minimum of moisture at which hair formation begins, an optimum in which the best development is obtained, and a maximum where the hair development nearly or entirely ceases. In his opinion, contact with soil particles is not a factor in the production of root hairs in soil. Support for this latter view (68, 97, 88, 13) as well as for the preceding one (90, 88) has been provided by the experimental results of a number of succeeding botanists.

It is a matter of general knowledge that roots of most plants produce hairs much more abundantly in moist air than in water. This is particularly obvious in corn, whose roots have a woolly appearance in moist air but become perfectly smooth on transfer to water. Mer (63), working with the roots of corn and of a number of other common crop plants, concludes that water suppresses root-hair production by stimulating root elongation. Other workers (82, 88, 96, 18) have attempted to explain decreased root-hair production in water on the basis of diminished oxygen supply. Their findings, though contradictory, suggest that more critical investigations along this line would yield interesting results.

The number of plants whose roots produce root-hairs normally in tap water is not large. To Bardell's list (3) of five common crop plants, namely, wheat, corn, squash, beans and peas, Farr (23) has added the names of approximately 80 more. However, Farr believes that many plants which have been reported as not producing root hairs in tap water will be found to do so when the proper adjustment is made of oxygen content, hydrogen ion concentration, osmotic pressure and chemical composition of the solution. Subsequent investigations by himself (24–33) and others (34, 12, 14) have supplied evidence to support this contention.

Temperature. Little reference has been made to the influence of temperature as a single factor on root-hair production. Snow (88) found that wheat roots in water produce numerous hairs between 4.5° and 11.5° C., fewer between 16.0° and 29.5° C. and none at higher temperatures. She concludes that high temperature with sufficient moisture tends to decrease hair production by increasing elongation of the internal cells. Jeffs (48), working with roots of radish and white mustard seedlings growing in a moist chamber, concludes that the hairs of these plants are extremely sensitive to changes of temperature when grown within the limits of 17° to 27° C. In watching the elongation of individual hairs he finds that a change of 3° to 5° C. will cause permanent cessation of growth in the hairs already formed.

Light. It would appear from the scanty data available (82, 88, 3, 48) that in most cases light as a single factor has no effect on root-hair production, provided other factors remain constant.

The roots of *Elodea canadensis* and those of a number of other water plants apparently provide an interesting exception. *Elodea* roots growing under normal conditions in water are hairless but develop hairs when they penetrate mud. As pointed out earlier, potential hair-producing cells (trichoblasts) occur in the epidermis of both water and soil roots.

Numerous investigators have tried to explain the hairless condition of *Elodea* roots in water and their haired condition in the soil. Schwarz (82) could arrive at no satisfactory explanation but does not think that the production of hairs in the soil is due to contact, chemical stimulation or retardation of the growth of the root. Snow (88), at the end of considerable experimentation, concluded that retardation due to contact with the soil particles is the principal factor. No further experimentation was reported until 1936 when Wilson (99) attempted to show that light is the controlling factor in the production of root hairs in *Elodea* roots. However, because of certain limitations in his light-proof apparatus, he is forced to conclude that the production of root hairs in *Elodea* is not affected by light but is bound up with some unknown stimulus which comes into play when the roots grow into the soil. A year later the present writer (13) published a paper on *Elodea* in which he reports that when light is completely excluded hairs are produced in water exactly as they are in the soil. Roots developed in water and in light are green and possess a cuticle, while those growing in darkness, whether in water or in soil, have neither of these peculiarities. When chlorophyll production is inhibited by means of ethylene gas, a cuticle is not formed, and hairs are plentifully produced in water even in light. The reviewer believes that the oxygen set free by photosynthesis in light is necessary for the formation of a cuticle which would prevent root-hairs from pushing out. In a recent paper Borgström (7) objects to Cormack's use of ethylene to "degreen" the water roots of *Elodea* growing in the light. According to Borgström, root-hair formation is an auxin response, and treatment with ethylene produces a transverse movement of auxins from the phloem to the epidermis. On the basis of this interpretation the break-down of chlorophyll in this case is considered by Borgström to be a simultaneous effect of the transverse hormone passage.

Still more recently King (50) has confirmed Cormack's observations as to the production of root hairs on water roots of *Elodea* growing in continuous darkness, and their absence in light. In his experiments the inhibition of root-hair production in the light could be overcome by adding small quantities of growth-promoting substances to the water. Only those compounds with the indole or naphthalene ring system and containing acid side chains are effective, and only the trichoblasts respond by forming hairs. He seems to favour Borgström's auxin theory of root-hair development and attributes the inhibition of root-hair formation in light to auxin utilization.

Whether root-hair development in *Elodea* is an auxin response or not must be left for further investigation to decide. At present it is not clear how the auxins influence the formation of a cuticle, and the exponents of the auxin theory of root-hair development have yet to explain why a trichoblast supposedly under the influence of auxins should form a hair, and an adjacent non-trichoblastic cell fail to do so.

Calcium. A great variety of chemical substances in solution have been applied to root hairs but few have yielded more interesting and instructive results than those which contain calcium. That calcium plays an important role in the normal development of all green plants is evidenced by the extensive literature that has accumulated in this regard. A general discussion of the importance of calcium does not lie within the scope of the present paper, and the reader is referred to a number of excellent reviews that have been written on this subject (75, 95, 2, 26).

Numerous workers (*e.g.*, 41, 42, 51, 57, 89) find that calcium is essential for root-hair production. Of these, two (41, 42, 51) have demonstrated most conclusively that root-hairs will not appear at all unless they receive a supply of calcium from outside the root. Mevius (64), on the contrary, has decided that in corn roots the presence or absence of root hairs is not associated with the presence or absence of calcium in the solution. It is now known (12), however, that corn roots are extremely sensitive to changes in pH, and, when this is properly adjusted, root hairs are densely developed in calcium solutions. The roots of bog plants show certain peculiarities in the development of root hairs which are found to be associated with their environment. Pearsall and Wray (67), working with the bog plant *Eriophorum angustifolium,* have shown that the root hairs of this plant tend to be longer and more abundant in solutions of low calcium content and high basic ratio.

Farr (23–33) and Farr (34–36) conclude at the end of a long series of experiments with the roots of Georgia collards that root hairs will not grow at all unless calcium is present in the surrounding solution and that it is used directly from this source for cell wall formation. In this connection the hydrogen ion concentration is shown to be an important factor. Acid solutions are found to have an inhibitory effect on root-hair growth which can, however, be overcome within limits by increasing the amount of calcium (28, 29, 32). In strongly alkaline solutions root hairs are poorly developed and the cortex is often ruptured. Since the literature on root-hair elongation has been fully covered by Farr (25, 33), attention may be called here only to new approaches to the problem in connection with developmental investigations.

The present writer (12) investigated the effect of the external factors, namely calcium and the pH of the solution on root-hair development. Microchemical tests revealed that calcium combines with pectic acid in the hardening of the elongating epidermal cell walls. Experiments with pure pectic acid *in vitro* show that this change does not take place in an acid medium and that in neutralizing the pectic acid there may be weak or strongly calcified pectates. These observations led to the conclusion that the amount of calcium and pH of the solution modify the rate at which calcification takes place.

In the roots of a number of the species investigated, namely, cabbage, white mustard, radish and Georgia collards, the epidermis is differentiated into rows of short and long cells (12). The long cells are found to be considerably more acid than the short cells in the hair-forming region of the root. Under most conditions the short cells are able to affect calcification of their cell walls at an early stage and thus form hairs. Through the gradual hardening of the cell walls vertical elongation is arrested, and, due to increasing pressure from within, the wall is pushed out at its softest point, invariably at or near the apical end of the cell. The long cells because of their acidity are not able to affect the change to calcium pectate at this time and thus remain hairless. Under conditions that facilitate more rapid calcification (excess calcium and a slightly alkaline medium), the long cells with their acidity neutralized also form hairs. Under conditions that bring about very rapid calcification (excess calcium and a strongly alkaline medium), the walls harden so rapidly that no hairs are produced, the epidermal cells become separated and their walls broken by the expanding contents. Under conditions that retard calcification (insufficiency of calcium and a slightly acid medium), the cells retain the pectic acid layer indefinitely and become greatly elongated, misshapen and swollen. Root hairs are abnormally branched and swollen or in extreme cases are absent. When roots growing under the latter set of conditions are transferred to a slightly alkaline calcium solution the importance of calcium for the hardening of the epidermal cell walls is clearly demonstrated. Immediate recovery, characterized by an obvious increase in the diameter of the root and by a sudden burst of long, straight, narrow hairs, always results. New hairs develop only in the region of new growth, and thus the deformed or hairless region above remains unchanged.

In the roots of other plants investigated (12, 14, 15), corn, tomato, endive and *Sporobolus,* the epidermis is not differentiated into long and short cells. All the cells are of one kind, and, although they are all long, they vary in length in the same region under the same conditions. Roots of this type are extremely sensitive to changes in pH, and in general they either are hairless or produce hairs sparsely in an aqueous medium. For example, corn roots which are densely haired in moist air become perfectly smooth on transfer to water or to neutral solutions of a calcium salt. When the solution is made decidedly alkaline, normal root-hair production results. Through this treatment the acidity of the epidermal cells, due doubtless to anaerobic respiration, is neutralized and the calcium ions thus made available to unite with pectic acid. Tomato roots also produce hairs sparsely in aqueous solutions. In this instance it is only under limited conditions, close to the neutrality point, where calcification takes place progressively but rapidly, that a dense production of hairs results. As mentioned already, in this root the response to changes in the environment is marked by a definite relationship between epidermal cell-length and length of the full-grown hair. The longest cells are either hairless or produce merely papillae, while the shortest cells produce the longest hairs.

The external factors not only determine the capacity of epidermal cells to produce root hairs but determine also the shape and length of the hairs themselves. In regard to the latter a number of workers have investigated abnormalities of root-hair growth under more or less abnormal conditions.

Stiehr (91) and others (101, 102, 76) obtained various types of abnormal growth under abnormal conditions. Hill (45) found branched and swollen hairs to be normal in the roots of certain marsh plants. Jeffs (48) observed the swelling of root hairs in response to changes in temperature. Farr, in his study of root-hair elongation, investigated this aspect of the problem in some detail. He (30, 32, 33) found that in Georgia collards variously deformed hairs are developed in dilute solutions, in solutions near the acid and alkaline limit for the growth of root hairs and when roots are transferred to solutions after growth in air has begun. Changes in root-hair morphology produced under these conditions are related to changes in the extent of the growing area at the tip of the hair and to changes in the direction of growth. More recently the present writer (12) obtained a great variety of branched, swollen and stunted hairs by growing cabbage roots in solutions deficient in calcium, and in solutions designed to render unavailable the calcium contained in the root itself. The abnormalities produced under these conditions strongly suggest the action of internal pressure on a wall that has been unequally or insufficiently hardened. The marked cessation of deformed hairs and the sudden development of long straight ones which always results on transfer of affected roots to calcium solutions substantiate this viewpoint.

Debraux (18) does not agree and asserts that such abnormalities are not due to the absence of calcium but are probably due to the presence of toxic substances secreted by the root itself. This criticism does not consider the possibility of the toxic substances rendering the calcium ions unavailable to the growing hairs. Neither does it take into account the marked cessation of deformities that occurs when the deformed roots are transferred to a calcium solution. Her own results, obtained from a study of radish roots grown in a variety of aqueous solutions, are rather difficult to interpret. She apparently associates deformity of the hairs with the presence of sugar or a salt in the aqueous medium. However, in considering the high concentrations used, it would appear that in this case the abnormalities are due rather to the high concentration of the medium which, as others (82, 101, 102, 91, 88, 33, 80) have shown, has a retardative effect upon root-hair elongation.

Growth Hormones. From the recognized fact that growth-promoting substances influence cell elongation (20, 44, 10), it is not surprising that such compounds should be applied to root hairs. A number of workers (60, 65, 93) have treated root hairs with growth hormones, but as yet there have been very few investigations of a developmental nature.

A noteworthy step in this direction has been made by Borgström (6). He grew roots of *Allium fistulosum* and those of a number of other common garden plants on agar containing small amounts of indole acetic acid under sterile conditions in test tubes. After a number of weeks the roots became decidedly swollen and entirely covered with hairs. Root hairs caused by artificial supply of growth hormones appeared along the entire root to the very tip. Root-hair development appears to be related to an auxin optimum, and the ranges within which hairs appear vary for each species. The optimum for roots of *Allium fistulosum* is 0.1 ppm. Higher concentrations almost entirely suppress root hairs. These observations are held by Borgström to support his assumption that normal root-hair production in nature is an auxin response and that the increased root-hair development induced by ethylene treatment is caused by growth hormones which are transported transversely from the phloem to the epidermis. Certain experiments regarding the use of ethylene to stimulate root-hair production in *Elodea* roots already have been referred to (13, 50).

Further studies on the influence of growth substances on root-hair development are needed. In the writer's opinion such work should be directed to the determination of the relationship between auxin-action and calcification of the epidermal cell walls and the investigation of morphological changes to the movement of food materials to the surface cells.

Internal Factors. With the exception of a number of references to the position of the nucleus (39, 100, 33) and to certain inherent qualities of the protoplasm (86, 4), very little consideration has been given to the influence of internal factors on the development of root hairs.

A beginning in this direction has been made by the present writer in an effort to discover the cause of differentiation of the epidermis of white mustard roots into long and short cells (16). As already mentioned, the long cells vacuolate early and do not ordinarily produce root hairs, while the short cells vacuolate much later and to a lesser extent and usually produce hairs. A study of transverse sections through the meristem of these roots reveals that each short cell is situated in direct line with a row of intercellular spaces within the cortex, while the long cells are not associated with intercellular spaces at any time of their development. On the basis of these observations and of data from Priestley's work (70, 71) on the nutrition of meristematic cells, these radiating rows of intercellular spaces are believed by the writer to facilitate the more rapid movement of soluble materials from differentiating vascular strands to the short cells. This hypothesis gives a reasonable explanation of this ability of the short epidermal cells to remain richly protoplasmic long after the long epidermal cells have become highly vacuolated. It is noteworthy that in roots of tomato and corn, where radiating rows of spaces either do not exist or do not extend completely through the cortex, all the epidermal cells are alike and all begin to vacuolate at a point much closer to the root apex than do the short epidermal cells of white mustard roots. A later note (17) reports the presence of a substance in these intercellular spaces capable of reducing alkaline silver.

Although the roots of most plants develop root hairs more abundantly under certain conditions than under others, avocado roots constitute an interesting exception. According to Burgis and Wolfe (9), the roots of this plant are hairless in humid air, sand, peatmoss, sand-peat mixture, water and nutrient solutions. A brown coloured periderm which develops two millimetres behind the white root tip is assumed to be responsible for the hairless conditions of avocado roots. Another interesting observation has been recorded recently by LaRue (55). According to him, very young hypocotyls of tomato, radish and cabbage seedlings, when deprived of their roots, produce outgrowths identical with root hairs. These hairs bear no resemblance to ordinary hairs of stems and leaves, and in no instance are they observed on normal unwounded seedlings.

From the data reported above it is evident that the production of root hairs is greatly affected by external conditions. Root hairs are produced within a wide range of temperatures, and, with the exception of the roots of a number of water plants, light has little or no effect. In general, root hairs are more densely developed in moist air than in water, although production may be increased in the latter by adjusting the oxygen content, hydrogen ion concentration and chemical composition of the solution. For the normal production of root hairs in aqueous solutions the importance of calcium and a suitable pH is indisputable. Calcium is essential for the hardening of the outer layer of the growing cell walls, and the pH regulates the rate at which this hardening process takes place. Recent studies with growth-promoting substances (auxins) give promise of interesting results. At present, available data on the precise action of auxins are so meagre that positive conclusions are difficult to draw.

THE MECHANISM OF ROOT-HAIR DEVELOPMENT

Two general theories have been advanced as to the mechanism of root-hair development. One, suggested by Snow (88), postulates that when vertical elongation of the epidermal cell is checked either by cessation of growth on the part of the inner cells or by external mechanical means, the cell extends horizontally to form a hair. Jeff's data (48) strongly support this theory. The second, advanced by Roberts (78) and already discussed in some detail, suggests that root hairs are evaginations produced by internal pressure on weaker portions of an unequally hardened cell wall. This idea was formulated at a time when there was considerable disagreement as to the exact difference between the hypothetical soft area and the rest of the cell wall. While different ideas have been advanced on this point, it is now fairly well established that the wall is more plastic at the apical end of an epidermal cell where a hair begins to push out. It is evident that these two theories are not fundamentally contradictory. In fact, the hardening or decrease in plasticity of the elongating epidermal cell walls may be the cause of the retardation of vertical elongation, and the increase in internal pressure which results may be the cause of the original swelling of the external wall. This last-mentioned point is the present writer's contribution to the discussion. Evidence has been obtained by him showing that the hardening process is associated with the incorporation of calcium into the outer wall layer of the epidermal cells and that the capacity to form hairs is dependent upon the rate at which calcification takes place. If calcification takes place gradually, and at the same time the cell contents begin to vacuolate, then the more plastic apical end of the epidermal cell will grow out sideways to form a hair; if calcification occurs too slowly, the cell will continue to elongate longitudinally and thus no hair will develop, calcification taking place after the cell has become completely vacuolated; and if it occurs rapidly before the cell contents have begun to vacuolate, no hair will form; or if rapidly at the same time that the cell contents are actively vacuolating, then the outside wall will break.

In the light of the above mentioned work the necessity of a thorough knowledge of the development of the epidermal cells as a basis for interpretation of root-hair formation becomes evident, as also the necessity of an acceptable explanation of the intercellular relationships of growing cells. While details of this work will not be dealt with here, I should like to mention briefly the opinions concerning certain aspects which can be considered pertinent to the problem in hand.

Priestley and a number of his students (70–74) have made a special study of changes in intercellular relationships in apical meristems. According to his investigations, intercellular readjustments are brought about by a gradual mutual adjustment of all cell walls "as a common framework" and not by the movement or sliding of one cell past another as contended by Krabbe (52). To this manner of intercellular adjustment he has proposed the term "symplastic growth". Sinnott and Bloch (87) have made a thorough study of growing epidermal cells in grass root tips. By watching the growth of small groups of surface cells they too conclude that the elongating cells do not slide along one another, but are held so firmly together that points in adjacent cell walls which are originally opposite each other are still opposite after these cells have greatly elongated. Moreover, they have shown that in individual cells the end of the cell toward the base of the root attains its final size sooner than the apical end of the same cell. Thus, according to these writers, changes in intercellular relationships are brought about by unequal growth of various portions of the cell wall in the same cell, and are referred to by them as "differential wall growth". In general, this theory closely resembles the "symplastic" concept of Priestley in that both conceive of intercellular adjustments as occurring within the common framework of cell walls, and do not involve sliding. Brumfield (8) has confirmed Sinnott and Bloch's observations in a similar study of grass root tips. By watching the growth of individual cells he observed that the basal end of an epidermal cell stops growing first, the middle part next and the apical end last, the cessation of growth passing along the cell from base to apex. In the meantime Meeuse (62), on the basis of an extensive investigation involving a wide variety of plant material, had concluded that changes in intracellular relationships are principally brought about by "symplastic" readjustments of the cells as as a whole, associated with cell expansion. A study of intercellular relationships has been carried out by the present writer (15) using the favourable material provided by the epidermis of tomato root tips. Careful measurements have shown that the cells are still growing in the region where the papillae first make their appearance and that the apical end of an individual cell continues to grow after the basal end has stopped. Moreover, it was shown that those parts of adjacent cells opposite the slowly growing basal portion of an individual cell do not grow so fast as those parts opposite the faster growing apical end of the same cell. The fact that different portions of an epidermal cell grow at different rates, *i.e.*, have differential cell wall growth, is believed by the writer to explain the greater plasticity of the cell wall at the apical end where a hair begins to push out and to account for the more yielding nature of the tip of the hair, once that structure is formed. Some evidence has been brought forward to show that although individual cells grow differentially, all the surface cells in the same region grow together and at a uniform rate (12, 14, 15). For instance, in roots with alternating zones of longer and shorter cells, all the cells in each zone are noticeably longer or shorter than those in the zone either directly above or below. Also in roots where zones of longer and shorter cells are produced experimentally by suddenly changing the environmental conditions, all the cells at the same level react at the same time to the new conditions.

Borgström (6, 7) suggests a third theory in this field. He considers that root hairs develop as soon as a certain amount of auxins reaches the external wall. Thus conditions which favour an increased transverse movement of auxins will stimulate root-hair formation. According to Borgström, his theory is not at variance with the two older theories of Snow and Roberts but represents an attempt to explain them on a hormone basis. In his opinion the transverse hormone stream causes a lateral growth which not only accounts for the simultaneous decrease in vertical elongation but also accounts for the external cell wall acquiring a softer consistency, since it is the endpoint of the transverse hormone stream. Confirmation or refutation of Borgström's theory must await the accumulation of information concerning the precise action of growth hormones on expanding cell walls. Some evidence of this kind has accumulated already (20, 10, 79, 11, 22), but it should be emphasized that the task is only begun. Whatever modifications have to be applied to Borgström's interpretation, the idea is a stimulating one and suggests the probable trend of future studies in this field.

In summing up, there is general agreement that root hairs result from retardation in vertical elongation of the epidermal cells. Although there is some difference of opinion as to how this occurs, the evidence in favour of a change in the physical and chemical composition of the elongating cell walls seems most convincing. There is general agreement that changes in intercellular relationships in the root epidermal result not from "sliding growth" but from differing rates of growth in different parts of the cell wall of individual cells and with all cell walls operating as one "common framework". If we accept the evidence that epidermal cells grow differentially, then an epidermal cell may be regarded as an ordinary parenchyma cell that has the capacity of changing its direction of growth. Whether it does so and grows into a hair, or whether it continues to elongate vertically and is hairless, will depend upon the conditions which prevail while the cells are actively vacuolating.

CONCLUSION

The study of root hairs and the superficial cells from which they arise has attracted many workers, partly no doubt because of their absorptive function and partly because their superficial position and relatively simple structure make them convenient for the study of cell enlargement. The object of the present paper has been to review the literature in this field from the developmental standpoint. To this end, most of the older data concerning root-hair development have been re-examined, and the observations of the earlier workers re-interpreted in the light of the results of more recent studies. Recent investigations have led almost invariably towards a greater exploration of both epidermal and root-hair cell walls. The series of papers which have appeared dealing with the microchemistry of the epidermal cell walls have cleared up many troublesome points. Even though our knowledge of the chemical composition of the cell wall and the way in which the cell wall grows is far from complete, it is certain that the present emphasis on this aspect of the problem is a sound and fundamental approach. The facts and principles here set forth have been formulated from observations made of developing superficial cells. How far they are applicable to other developing cells of the thin-walled parenchymatous type remains to be seen.

LITERATURE CITED

1. Addoms, Ruth M. Toxicity as evidenced by changes in the protoplasmic structure of root hairs of wheat. Amer. Jour. Bot. **14**: 147–165. 1927.
2. Arrhenius, O. Kalkfragen, Bodenreaktion und Pflanzenwachstum. 1926.
3. Bardell, E. M. The production of root hairs in water. Univ. Washington, Publ. Bot. **1**: 1–19. 1915.
4. Bloch, R. Differentiation in red root-tips of *Phalaris arundinacea*. Bull. Torrey Bot. Club **70**: 182–183. 1943.
5. Bonner, J. The chemistry and physiology of the pectins. Bot. Rev. **2**: 475–497. 1936.
6. Borgström, G. Root hair formation as an auxin response. Kungl. Fysiografiska Sällskopets 1 Lund Förhandlingar **9**: 1–8. 1939.
7. ————. The transverse reactions of plants. 230 pp. 1939.
8. Brumfield, R. T. Cell growth and division in living root meristems. Am. Jour. Bot. **29**: 533–543. 1942.

9. Burgis, D. S. and Wolfe, H. S. Do avocado roots develop root-hairs? Proc. Fla. State Hort. Soc.

10. Burgström, H. The influence of heteroauxin on cell growth and cell development. Lantbrukshögskolans Annaler **10**: 209–240. 1942. Biol. Abs. **17**: 1972–1973. 1943.

11. Commoner, B. *et al.* The mechanism of auxin action. The effect of auxin on water absorption by potato tuber tissue. Am. Jour. Bot. **30**: 23–28. 1943.

12. Cormack, R. G. H. Investigations on the development of root hairs. New Phytol. **34**: 30–54. 1935.

13. ————. The development of root hairs by *Elodea canadensis*. New Phytol. **36**: 19–25. 1937.

14. ————. The effect of environmental factors on the development of hairs in *Phleum pratense* and *Sporobolus cryptandrus*. Am. Jour. Bot. **31**: 443–449. 1944.

15. ————. Cell elongation and the development of root hairs in tomato roots. Am. Jour. Bot. **32**: 490–496. 1945.

16. ————. A comparative study of developing epidermal cells in white mustard and tomato roots. Am. Jour. Bot. **34**: 310–314. 1947.

17. ————. A substance in the intercellular spaces of white mustard roots that reduces alkaline silver. Canad. Jour. Res., C., **26**: 263–268. 1948.

18. Debraux, Germaine. Influence de diverses substances dissoutés sur la morphologie et la biologie des poils radicaux. Rev. Gén. Bot. **50**: 378–395. 1938.

19. Devaux, H. Action de la lumière sur les racines. Bull. Soc. Bot. France. **35**: 305–308. 1888.

20. Diehl, J. M. *et al.* The influence of growth hormone on hypocotyls of *Helianthus* and the structure of their cell walls. Rec. Trav. Bot. Néerl. **36**: 709–798. 1939.

21. Dittmer, H. J. A critical study of subterranean plant parts. Am. Jour. Bot. (Abs.) **33**: 819. 1946.

22. Eyster, H. C. Auxin action. Science **97**: 358–359. 1943.

23. Farr, C. H. The formation of root hairs in water. Proc. Iowa Acad. Sci. **32**: 157–165. 1925.

24. ————. Root-hair elongation in Knop's solution and in tap water. Am. Jour. Bot. **12**: 372–383. 1925.

25. ————. Studies on the growth of root hairs in solutions. 1. The problem, previous work and procedure. Am. Jour. Bot. **14**: 446–456. 1927.

26. ————. Studies on the growth of root hairs in solutions. 2. The effects of concentrations of calcium nitrate. Am. Jour. Bot. **14**: 497–515. 1927.

27. ————. Studies on the growth of root hairs in solutions. 3. The effects of concentrations of $CaCl_2$ and $Ca(OH)_2$. Am. Jour. Bot. **14**: 553–564. 1927.

28. ————. Studies on the growth of root hairs in solutions. 4. The pH-molar-rate relation for collards in calcium chloride. Am. Jour. Bot. **15**: 6–31. 1928.

29. ————. Studies on the growth of root hairs in solutions. 5. Root-hair elongation as an index of root development. Amer. Jour. Bot. **15**: 103–113. 1928.

30. ————. Studies on the growth of root hairs in solutions. 6. Structural responses to toxic pH and molar concentrations of calcium chloride. Am. Jour. Bot. **15**: 171–178. 1928.

31. ———. Studies on the growth of root hairs in solutions. 7. Further investigations on collards in calcium hydroxide. Bull. Torrey Bot. Club 55: 223–246. 1928.
32. ———. Studies on the growth of root hairs in solutions. 8. Structural and intracellular features of collards in calcium nitrate. Bull. Torrey Bot. Club 55: 529–553. 1928.
33. ———. Root hairs and growth. Quart. Rev. Biol. 3: 343–376. 1928.
34. Farr, Wanda K. The elongation of root hairs in solutions of single calcium compounds. A comparison of three species, *Brassica oleracea, Avena sativa, Oryza sativa.* Am. Jour. Bot. 14: 627. 1927.
35. ———. The pH-molar rate relation for collards in calcium sulphate. Am. Jour. Bot. 15: 616. 1928.
36. ———. The elongation of root hairs of *Brassica oleracea* in solution of primary calcium phosphate. Am. Jour. Bot. 16: 852. 1929.
37. ———. Cell walls and synthetic fibers. Eco. Bot. 1: 98–113. 1947.
38. Girton, R. E. The growth of citrus seedlings as influenced by environmental factors. Univ. Cal. Publ. Agr. Sci. 5: 83–117. 1927.
39. Haberlandt, G. Ueber die Lage des Kernes in sich entwickelenden Zellen. Ber. Deut. Bot. Ges. 5: 205–212. 1887.
40. ———. Physiological plant anatomy. Ist Ed. 1914.
41. Hansteen, B. Über das Verhalten der Kulturpflanzen zu den Bodensalzen. 1, 2. Jahrb. Wiss. Bot. 47: 289–376. 1910.
42. Hansteen-Cranner, B. Über das Verhalten der Kulturpflanzen zu den Bodensalzen. 3. Jahrb. Wiss. Bot. 53: 536–598. 1914.
43. Hayward, H. E. and Blair, Winifred M. Some responses of Valencia orange seedlings to varying concentrations of chloride and hydrogen ions. Am. Jour. Bot. 29: 148–155. 1942.
44. Heyn, A. N. J. The physiology of cell elongation. Bot. Rev. 6: 515–574. 1940.
45. Hill, T. G. Observations on the osmotic properties of the root hairs of certain salt-marsh plants. New Phytol. 7: 133–142. 1908.
46. Hoppmann, O. Über das Vorkommen und die Mikrochemie des Amyloids in pflanzlichen Zellhauten. Inaug. Diss. Münster. 1930.
47. Howe, Caroline G. Pectic material in root hairs. Bot. Gaz. 72: 313–320. 1921.
48. Jeffs, R. E. The elongation of root hairs as affected by light and temperature. Am. Jour. Bot. 12: 577–606. 1925.
49. Kerr, T. The injection of certain salts into the protoplasm and vacuoles of the root hairs of *Limnobium spongia.* Protoplasma 18: 440. 1933.
50. King, L. J. Responses of *Elodea densa* to growth-regulating substances. Bot. Gaz. 105: 127–151. 1943.
51. Kisser, J. Über das Verhalten von Wurzeln in feuchter Luft. Jahrb. Wiss. Bot. 64: 416–439. 1925.
52. Krabbe, G. Das gleitende Wachstum bei der Gewebebildung der Gefässpflanzen. 1886.
53. Kraemer, K. Wurzelhaut, Hypodermis und Endodermis der Angiospermwurzel. Bib. Bot. Heft, 59. 1903.
54. Küster, E. Ueber die Beziehungen der Lage des Zellkernes zur Zellenwachstum und Membranbildung. Flora 97: 1–23. 1907.
55. LaRue, C. D. Root hairs on stems and leaves. Am. Jour. Bot. (Abs.) 33: 823. 1946.
56. Leavitt, R. G. Trichomes of the root in vascular cryptogams and angiosperms. Proc. Boston Soc. Nat. Hist. 31: 273–313. 1904.
57. Magowan, Florence N. The toxic effect of certain common salts in the soil on plants. Bot. Gaz. 45: 45–49. 1908.

58. McCoy, Elizabeth. Infection by *Bact. Radicicola* in relation to the microchemistry of the host's cell walls. Proc. Royal Soc., B, **110:** 514–533. 1932.
59. McDougall, W. B. Thick-walled root hairs of *Gleditsia* and related genera. Am. Jour. Bot. **8:** 171–176. 1921.
60. Meesters, A. The influence of hetero-auxin on the growth of root hairs and roots of *Agrostemma githago* L. Kung. Akad. Wet. Amsterdam, Proc. Sect. Sci. **39:** 91–97. 1936. Biol. Abs. **11:** 1958. 1937.
61. Meeuse, A. D. J. Development and growth of the sclerenchyma fibers and some remarks on the development of the tracheids in certain monocotyledons. Rec. Trav. Bot. Néerl. **35:** 288. 1938.
62. ————. A study of intercellular relationships among vegetable cells with special reference to "sliding growth" and to cell shape. Rec. Trav. Bot. Néerl. **38:** 18–143. 1941.
63. Mer, E. Recherches expérimentales sur les conditions de developpement des poils radicaux. Compt. Rend. Acad. Sci. Paris **88:** 665–668. 1879.
64. Mevius, W. Kalzium—Ion und Wurzelwachstum. Jahrb. Wiss. Bot. **66:** 183–253. 1926.
65. Miller, E. C. Plant physiology. 2nd Ed. 1201 pp. 1938.
66. Parker-Rhodes, A. F. Preliminary experiments on the estimation of heteroauxin in soils. Jour. Agr. Sci. **30:** 654–671. 1940.
67. Pearsall, W. H. and Wray, E. M. The physiology and ecology of the calcifuge habit in *Eriophorum angustifolium*. Jour. Ecol. **15:** 1–32. 1927.
68. Pfeffer, W. Zur Kenntniss der Plasmahaut und der Vacuolen nebst Bemerkungen über den Aggregatzustand der Protoplasmas und über osmotische Vorgänge. Abh. Sächs. Ges. Wiss. **16:** 185–197. 1889.
69. Pinkerton, M. Elizabeth. Secondary root hairs. Bot. Gaz. **98:** 147–158. 1936.
70. Priestley, J. H. The meristematic tissues of the plant. Biol. Rev. **3:** 1–20. 1928.
71. ————. Cell growth and cell division in the shoot of the flowering plant. New Phytol. **28:** 54–81. 1929.
72. ————. Studies in the physiology of cambial activity. 1. Contrasted types of cambial activity. New Phytol. **29:** 56–73. 1930.
73. ————. Studies in the physiology of cambial activity. 2. The concept of sliding growth. New Phytol. **29:** 96–140. 1930.
74. ———— and Scott, Lorna I. The formation of a new wall at cell division. Proc. Leeds Phil. Lit. Soc. **3:** 532. 1939.
75. Reed, H. S. The value of certain nutritive elements to the plant cell. Ann. Bot. **21:** 502–543. 1907.
76. Reinhardt, M. O. Das Wachstum der Pilzhyphen. Ein Beitrag zur Kenntniss des Flächenwachstums vegetabilischer Zellmembranen. Jahrb. Wiss. Bot. **23:** 479–566. 1892.
77. Ridgway, C. S. The occurrence of callose in root hairs. Plant World **16:** 116–122. 1913.
78. Roberts, Edith A. The epidermal cells of roots. Bot. Gaz. **62:** 488–506. 1916.
79. Ruge, U. Zur Theorie der Mechanik der Zellstreckung und des Streckungs-Wachstums. Planta **32:** 571–584. 1942.
80. Sakamura, T. and Kanamori, H. Ueber die Wirkung der Essigsäure, des Ammoniaks und ihrer Salze auf das Protoplasma des Wurzelhaares. Jour. Fac. Sci. Hokkaido Imp. Univ. V. **4:** 65–98. 1935.
81. Sauvageau, C. Sur la racine du Najas. Jour. Bot. **3:** 3–11. 1889.

82. SCHWARZ, F. Die Wurzelhaare der Pflanzen. Unters. Bot. Inst. Tübingen **1**: 135–188. 1883.
83. SEIDEL, K. Untersuchungen uber das Wachstum und die Reizbarkeit der Wurzelhaare. Jahrb. Wiss. Bot. **63**: 501–552. 1924.
84. SIFTON, H. B. Developmental morphology of vascular plants. New Phytol. **43**: 87–129. 1944.
85. SINNOTT, E. W. Growth and differentiation in living plant meristems. Proc. Nat. Acad. Sci. **25**: 55–58. 1939.
86. ———— and BLOCH, R. Cell polarity and the differentiation of root hairs. Proc. Nat. Acad. Sci. **25**: 248–252. 1939.
87. ———— and ————. Changes in intercellular relationships during the growth and differentiation of living plant tissues. Am. Jour. Bot. **26**: 625–634. 1939.
88. SNOW, LAETITIA M. The development of root hairs. Bot. Gaz. **40**: 12–48. 1905.
89. SOROKIN, HELEN and SOMMER, ANNA L. Changes in the cells and tissues of root tips induced by the absence of calcium. Am. Jour. Bot. **16**: 23–39. 1929.
90. SPALDING, V. M. Biological relations of certain desert shrubs. 1, The creosote bush (*Covillea tridentata*) in its relation to water supply. Bot. Gaz. **38**: 122–138. 1904.
91. STIEHR, G. Das Verhalten der Wurzelhaare gegen Lösungen. Diss. Kiel. 1903.
92. SUCHARIPA, R. Protopectin and some other constituents of lemon peel. Jour. Am. Chem. Soc. **46**: 145–146. 1924.
93. SWEENEY, BEATRICE M. The effect of auxin on protoplasmic streaming of root hairs of *Avena*. Am. Jour. Bot. **31**: 78–80. 1944.
94. TIEGHEM, PH. VAN. Sur les poils radicaux geminés. Ann. Soc. Nat. Bot. VII. **6**: 127–128. 1887.
95. TRUE, R. H. The significance of calcium for the higher green plants. Science **55**: 1–6. 1922.
96. VÖCHTING, H. Ueber die Keimung der Kartoffelknollen. Bot. Zeit. **60**: 87–114. 1902.
97. WENT, F. A. F. C. Ueber Haft- und Nahrwurzeln bei Kletterpflanzen und Epiphyten. Ann. Jard. Bot. Buitenzorg. **12**: 2–12. 1894.
98. WHITAKER, EDITH S. Root hairs and secondary thickening in the Compositae. Bot. Gaz. **76**: 30–59. 1923.
99. WILSON, K. The production of root hairs in relation to the development of the piliferous layer. Ann. Bot. **50**: 121–154. 1936.
100. WINDEL, E. Beziehungen zwischen Function und Lage des Zellkernes bei Wurzelhaare. Beih. Allg. Bot. **1**: 45–93. 1917.
101. WORTMANN, J. Beiträge zur Physiologie der Wachstum. Bot. Zeib. **47**: 277–286. 1889.
102. ZACHARIAS, E. Ueber das Wachstum der Zellhaut bei Wurzelhaaren. Flora **74**: 466–491. 1891.
103. ZEIGENSPEK, H. Das Amyloid jugendlicher Organe. Das Amyloid in den wachsenden Wurzelhaaren und seine Beziehungen zum Zellwachstum. Ber. Deut. Bot. Ges. **38**: 328–333. 1920.

CONTROL OF POLLINATION IN SUGAR-BEET

ALEXANDER ARCHIMOWITSCH

Formerly, Professor, Departments of Plant Culture, Agricultural Institutes, Bela Cerkov, Kiev, and Shitomir, USSR

Beginning in 1923 the writer has given attention to the biological processes concerned in the flowering and in the control of pollination in sugar-beet. Correct solutions of the problems involved have important bearing on sugar-beet genetics and will have influence on practical methods of selection and seed-breeding. If the essential processes that go on during the flowering and pollination of sugar-beet were known, possibility would be afforded to control the processes and to direct them. We could then either limit undesired hybridization to a large extent and hold pollination within a certain selected group or even among flowers of one plant (inbreeding), or, on the other hand, obtain other less restricted types of hybridization. The results of scientific investigations over a number of years on this subject are herewith summarized.

BIOLOGY OF SUGAR-BEET FLOWERING

The observations of early workers on flowering and pollination processes in sugar-beet were carried out without application of plant embryological methods, and these observations, therefore, gave only approximate and, as we shall see later, partly erroneous ideas about the phenomena. The chief defect arose in flowering of sugar-beet being considered for some time as protandrous (13, 15, 20, 40, 43, 47). Application of methods of plant embryology established the fallacy of this statement and proved that sugar-beet does not manifest protandry (10, 11, 17, 27, 30, 35, 36, 37). The flowering of sugar-beet lasts one month or even longer. Alternation in the opening of flowers on a plant and the opening and closing of individual flowers were described by Fruwirth (20) and later were investigated by the writer (1–4). It appears that the shoots on which flowers open earliest have better developed glomerules. The central flowers of an inflorescence open earlier and produce seeds that yield plants with superior roots. In their turn, better developed glomerules and roots give larger shoots and later stronger plants (9, 14, 38, 50, 51).

The agencies of pollen transfer are insects and wind. It is generally acknowledged that wind is of first importance. In order to learn the distance of spread of pollen in a horizontal direction, different investigators have utilized small glass plates covered with vaseline or glycerine which were set up at various distances from a beet plantation in flower (18, 42). Under direction of the writer, German, an instructor of the Agricultural Institute in Shitomir, for three years studied the horizontal spread of sugar-beet pollen. It appeared from his results that the pollen can be distributed at least 4,500 meters by wind.

In 1938 German (22) studied, with the help of airplanes, the spread of pollen in a vertical direction. It was found that pollen was present in the air at a height of 2,200 meters. The larger part was found up to a height of 750 meters. Higher than that, the quantity of grains in the air sharply diminished. In 1938 Meier and Artschwager (32) observed a still greater height of pollen carriage (5,000 meters). It is quite comprehensible that pollen grains, after having been lifted so high with the help of a vertical warm air stream, may spread very far in a horizontal direction.

The structure of the sugar-beet flower indicates that the importance of insects as agents of pollen dispersal is not to be overlooked. Some characteristics of sugar-beet flower structure that may seem to contribute to this phase are the clustering of the small flowers into groups, conspicuous flower stalks that can be seen from afar, secretion of nectar, the strong characteristic odor and the fact that the flower produces much nutritious pollen for the insects.

Furthermore, the structure of the beet flower is simple, and pollination is not restricted to particular insect types. Many kinds of insect visit sugar-beet flowers. Some occur in great numbers and are of predominant importance as agents of pollen transfer. In 1913 Uzel (45) and Shaw in 1914 (41) made observations on the insects which pollinate sugar-beet flowers. The writer also has given attention to the matter (1, 4). As a result of his investigations it can be said that in the Ukraine the insects that pollinate sugar beet flowers can be divided into groups as follows:

a) Insects that visit flowers only for the nectar that is secreted by the gland ring of the flower; for instance, the wasplike fly *Melithreptus scriptus* L.;

b) Insects which eat pollen, chiefly beetles of the genera *Zonabris, Leptura* and *Cerocoma;*

c) Insects which visit the flowers for nectar as well as for pollen, such as the common honey bee (*Apis mellifica* L.) and wild bees of the genera *Andrena* and *Halictus.* Honey bees apparently visit the flowering beet fields with some unwillingness. However, in years when they cannot find much nectar and pollen from other flowers they appear over the flowering sugar-beet plantations in large numbers. Such activity of honey bees was observed by Vasilie in the Ukraine in 1912 and by the writer at Belaya Zerkov, U.S.S.R., in 1925.

d) Insects that suck the sap from flower parts and from the green branches and leaves of the flower shoots. These are thrips; plant-lice, especially *Aphis fabae* Scop. (*papaveris* Fabr.); and a number of other insects (*Mesocerus, Palomena,* etc.)

e) Insects which are attracted to sugar-beet plants by plant lice. These are lady-bird beetles and their larvae (*Coccinella septempunctata*) and other species of this genus. Ants also visit the aphid colonies. Lady-bird beetles and their larvae are predaceous insects. As they hunt for plant-lice at the same time they serve as spreaders of pollen. The ants feed on the sweet secretion of plant-lice and incidental thereto may transfer pollen. In his earlier publications (1, 4) the writer has given a more detailed list of insects which pollinate sugar-beet flowers.

All of the three theoretically possible types of pollination, namely, autogamy, geitenogamy and xenogamy, may take place with sugar-beet. Autogamy occurs quite automatically, since when the flowers open, the pollen-sacs burst and the clumps of pollen grains are discharged on the inner parts of the flower. They therefore reach the stigmas of the pistil prior to any other pollen. Though the stigmas are not quite open there is a fissure between them, and the pollen grains may fall there and germinate. Harechko-Savitzky (27) observed that even 15 minutes after the opening of a flower, 13% of the pollen grains which have reached the stigmas are in a state of germination. In five hours the majority of pollen grains (94%) showed pollen tubes. Soon after the opening of flowers, the clumps of pollen grains dry, crumble into separate grains and are then distributed by wind or insects. As a result of this process there occurs either transport of pollen from one flower to another on the same plant (geitenogamy or self pollination) or transport of pollen from one plant to another one (xenogamy or cross pollination). But as a result of the self-sterility that prevails in sugar-beet, the fertilization that occurs is chiefly by xenogamy. The reason for this in sugar-beet, as well as in other auto-sterile plants, is that pollen tube growth on stigmas of the same plants is very weak. Fertilization by xenogamy, usually in the first 24 hours after the opening of a flower, is therefore accomplished by the faster growing pollen from some other plant. The experiments of Harechko-Savitzky in 1930 (27) place the time limits as 19–24 hours, but according to Oksijuk (37) fertilization may sometimes occur within 3½ hours after opening of the flower. Harechko-Savitsky (29) succeeded in hastening the growth of pollen tubes by changing temperature exposures. Ordinary varieties of sugar-beet are practically auto-sterile, but sometimes we find races among them that are highly auto-fertile. Hjalmar-Nilsson (34) first pointed out the existence of such races in 1922–1923, and later the writer found a great quantity of such races in the sugar-beets used in breeding work in the Ukraine. Especially noteworthy are the races noted by plant-breeder Grinko (23, 24) at the Ivanivska plant-breeding station (Kharkov district). The writer also succeeded in cultivating some auto-sterile races of sugar-beet. Studying the speed of growth in pollen tubes, Zajkovskaja (49) proved that the rate during self-pollination of auto-fertile races is much higher than that in auto-sterile races; in some races it approaches that in xenogamous pollination. As for heredity of auto-fertility, the writer (5) stated that self-fertility is the prevailing condition. Later work by Grinko (25) confirmed this statement.

CONTROL OF POLLINATION IN SUGAR-BEET

The methods of controlling pollination in sugar-beet can be classified on the basis of the isolation techniques employed or the type of manipulation given to the plant material.

Isolation Techniques

a) Isolation of separate plants or their parts for the purpose of obtaining seeds by self-pollination.

b) Isolation of pairs of plants for the purpose of restricting the crossing to two individuals, for example, closely related plants.

c) Isolation of little groups of plants for the purpose of increasing selected material.

d) Isolation of large groups of plants for the purpose of breeding varieties.

Manipulation of Plant Material

a) Hybridization under isolators with preliminary castration of flowers, followed by application of pollen from the chosen male parent.

b) Hybridization under isolators without anther removal or artificial pollination.

c) Hybridization by growing selected sorts together in a seed plot.

Isolation Techniques

a) Isolation of separate plants or their parts for the purpose of obtaining seeds by self-pollination.

Sugar-beets can be isolated from one another by distance or otherwise. Experiments on space isolation have been carried out by a great many investigators (12, 16, 20, 31), but in the light of the foregoing experiments on spread of pollen in the air, we cannot consider the results of these experiments as entirely trustworthy. It is clear that in large scale work, when a great number of plants must be isolated, the method of space isolation can hardly be utilized. Quite naturally, therefore, many investigators have paid attention to devising other methods (19, 33, 44, 46).

My own experiments in this direction began with trials and comparisons of different isolators as used by other investigators (2). It came out that the best of all these devices was one developed by Munerati (33) because it involves using both an isolation fabric and glass, a combination that greatly reduces etiolation of the enclosed plants. I have further improved this method by placing the glass not on the cover of the chamber but on the sidewall turned to the north. Thanks to this the plants were not overheated. According to suggestions by Prof. V. Savitsky, the isolators were made quite light and portable and so could be held up by a wire. We met with considerable difficulty in choosing the most suitable fabrics, for we did not have expensive cambric with 40 threads per centimeter at our disposal, but had to use cheap cotton (byas) with only 22 threads. The cotton threads, however, were rough and nappy and therefore the spaces between them were almost filled. Thanks to this, the average distance between naps was about 11.4 μ (with some deviations). The diameter of pollen grains varies usually from 15.9 to 22.5 μ, according to my observations (1); according to the observations of Oksijuk (35), from 15 to 23.4 μ; but according to the statements of Harechko-Savitsky (27), from 6 to 30 μ. It is quite clear, therefore, that the pollen of sugar-beet may be forced into the isolator by wind, and there arose, consequently, the necessity of studying the effectiveness of these isolators made from the cheap cotton cloth in keeping out pollen. Toward this end I had for some years been carrying on different experiments following a plan in which sugar-beet and red-garden beet plantations were arranged in checker board pattern. Each plant of sugar and of red-garden beet was enclosed separately in an isolator. Seeds were gathered separately from the sugar-beet plants of each planting. The seeds were sown, and the percentage of red-garden beet hybrids in the progeny was determined. The average quantity of hybrids in three years was, respectively, 15%, 0.4% and 1.3%. The true percentages were probably twice as great as these figures because our method afforded no way of distinguishing hybrids among sugar-beet plants but uncovered only hybrids between sugar and red-garden beets. But even taking this into consideration the results of our experiments are quite satisfactory for practical selectional work. During the isolation of separate plants with such a method I got in some cases by the isolation of self-fertile plants nearly 57 gr. or 4885 glomerules from one root.

For carrying out inbreeding on a large scale, isolation of whole plants is rather expensive and inconvenient. Hjalmar-Nilsson (34) isolated separate branches with the help of isolators made of parchment-paper. At first this method was considered free from criticism because parchment-paper is quite impenetrable by pollen, but examination of the method by Fruwirth (21) proved that the majority of plants in the progenies traceable to such isolations were hybrids. Experiments carried out by the author confirmed this (3). Meanwhile the parchment-paper isolators were set on tens of thousands of plants. It was therefore necessary to find a method of destroying the pollen which had penetrated from neighbor plants onto one to be isolated. The writer proposed for this purpose to wet the branches of the plants prior to putting on the bags. In that case foreign pollen grains were caused to rupture, and their capacity to germinate was thereby destroyed. By application of this precautionary measure, the percentage of hybrids in progenies was greatly diminished. If self-fertile plants were isolated, hybrids were not to be found at all (5, 7). The number of glomerules under one parchment paper isolator reached in some cases as high as 590.

b) Artificial isolation of two plants of common origin (sibs).

This method was proposed by Munerati (33). The advantage of it lies in the fact that it permits the breeder to carry out the inbreeding work not only with auto-fertile plants but also with auto-sterile ones. Our experiments showed that here the danger of undesirable crossings was less than by isolation of separate plants because the majority of glomerules formed was the result of the hybridization as directed. By utilization of the Munerati type isolators as improved by the writer, the percentage of undesirable hybrids in one experiment was approximately 0.47. Under such isolators, by isolation of sib pairs we got much higher seed yields than by isolation of separate plants.

c) Isolation of plants in small groups for the purpose of increasing the selected material.

In the literature concerning the breeding of sugar-beets unequivocal statements cannot be found regarding the distance that may be sufficient for space isolation in small groups of plants. On this question there are some data by Vilmorin (46) who thought that one kilometer cannot guard against undesirable crossings, and by Sundelin (42) who considered that the distance of 100–125 meters was, for practical purposes, quite sufficient for isolation of small group plantings.

In order to find the necessary distance that should be maintained between small groups of sugar-beet plants, the writer conducted experiments over a three-year period. In the middle of the field occupied with other crop plants there were planted from 300 to 600 red garden beet (Egyptian) roots. Along radii in eight directions (N, NE, E, SE, S, SW, W, NW) and at different distances from the center, small groups of sugar-beets were planted. A study of progenies from the various plantings permitted determination of the percentage of sugar x red garden beet hybrids for each location. As we expected, it turned out that the percentage of hybrids varied among the different directions chosen for the plantings. The deviations were attributable to direction and strength of the wind during the period of flowering as well as to other meteorological factors, chiefly precipitation. The results of this experiment were described in one of the writer's reports (3).

The data of these experiments carried out during the three years are summarized in Table 1. It is recognized that specific percentages may be taken only as representing approximate data to be expected under somewhat similar circumstances and that in each case there may exist some variations. However, as one thing that may be stated for all cases, in carrying out the practical work with selected sugar-beets we may accept 200 meters as a limiting distance between small group plantings. On the other hand, to separate group plantings at such a distance from other sugar-beet plants is not always convenient or economical. Therefore, the groups are very often placed at distances of 5, 10 and 20 meters and are separated from each other by plants of greater height, *e.g.*, hemp, or, in the South, sorghum, such as Sudan grass.

TABLE 1

HYBRIDIZATION OCCURRING IN AN EXPERIMENT IN WHICH RED GARDEN-BEETS AS A POLLEN SOURCE WERE SURROUNDED, AT DISTANCES RANGING FROM 0 TO 200 METERS, BY SUGAR-BEETS. (RESULTS GIVEN AS AVERAGE FOR THREE YEARS OF TEST).

Distance from central part in meters	Average percentages of hybrids	Variations
	%	%
From 0–80 meters	7.7	0–24
" 80–200 "	1.2	0– 4.1
" 200 and more	0.3	0– 9

Naturally there arises the question whether such separation with plant screens has advantageous effects. During three years work under the writer's direction at the Agricultural Institute of Shitomir, German carried out some experiments to test the matter. Group plantings of sugar-beet and red garden-beet (25 plants in each group) were placed in a checker board order at distances of 6, 7, 10 and 12 meters one from another. The spaces between groups were sown with Italian hemp which grows to considerable height. Test of progenies obtained from these groups gave the results shown in Table 2. These data show that in some years a distance of 12 meters between groups was sufficient to eliminate cross pollination if the areas between the groups of plants were sown with plants capable of forming efficient screens. But it is not possible to foresee all the meteorological conditions that may prevail during the flowering of sugar-beets.

A better guarantee against undesirable crossing among groups of plantings is afforded by other means of isolation. Such experiments have been carried out by many investigators, but each time they got very poor seed yields. This failure cannot be attributed wholly to auto-sterility because frequently not only individual plants but also groups of plants were isolated. It is possible that failure of seed to set is a result of unfavorable conditions under isolators for formation and development of glomerules. Therefore, there arose the necessity of studying these condtions. Temperature and relative humidity under isolators have been studied by three investigators (12, 39, 48).

TABLE 2

EFFECTS OF PLANT SCREENS GROWN BETWEEN SUGAR-BEET AND RED GARDEN-BEET IN PREVENTING HYBRIDIZATIONS.

Year of test	Distance between the sugar beet and red garden beet groups	Average percent of hybrids in progenies
1st year	6 meters	17.12
2nd year	10 meters	5.45
3rd year	12 meters	0.72

The efforts of the writer and his collaborators were also dedicated to this problem. In 1934 we studied the microclimate under the isolators, using isolators of our own construction of such size that from six to nine plants could be covered. Thermographs and humidity recorders were placed in these isolators (17). The experiments showed that the condtions inside the isolators were very unsuitable for growth and development of the plants. On some hot and humid days the temperature reached 44.5° C. At the same time relative humidity of the air inside the isolators was considerably lower than that outside. Owing to these facts the writer had to invent a new type of isolator in which these undesirable conditions would be removed. As a result of experiments extending over many years an isolator was constructed in which the temperature and humidity of the air remained almost normal, that is, they did not differ very much from the temperature and humidity of the air outside the isolator. This construction was as follows: a small frame was built, three walls of which were covered with cotton cloth. The wall that was to be turned to the north and the ceiling were made of glass. The height of the chamber was approximately 190 cm., the width and length approximately 250 cm. Inside the chamber there was room for planting nine mother beets. Lowering of temperature was obtained by means of water conducted in rubber tubes on a platform above the isolator from a tank and allowed to trickle in a thin layer over the isolator's sloping glass ceiling. The slight slope was from north to south. The water was allowed to run down the cotton stuff of the south wall of the cage and make it wet. The sheet of water on the glass ceiling not only lowered the temperature inside the isolator but also absorbed the infrared rays of the sunlight and thus prevented overheating of air inside the isolator. The cotton side wall which was turned to the south and which was the most heated by the sunshine remained wet all the time. In order to increase the evaporation of water from this wall a stream of air was directed on it all the time by means of small portable electric fans inside the isolators. The evaporation assisted in cooling the air inside the isolator, and the air set in motion inside the isolator helped in transferring pollen from one plant to another. Details of construction and of the experiments have been published (8, 9).

Testing of these isolators was carried out in 1937. They were found very effective in decreasing temperature and in raising relative humidity inside them. For example, during the investigations the inside temperature (with cooling) at 1 p.m. was only 1.3°C more than the oustide temperature. At the same time the temperature of air in control isolators of the same construction, but without cooling, was 7.9°C higher than the temperature of outside air. Additional interesting data were obtained on hot days. For instance, at 1 p.m. on July 26, 1937, the temperature of outside air reached 27.3°C, the temperature inside the isolator with cooling was 30.8°C, and the temperature inside the control isolator without any cooling reached 44.9°C. Therefore the temperature inside the isolator with cooling was 14.1° lower than that of the isolator without cooling. As regards the humidity of the air it can be stated that in control isolators, *i.e.*, without cooling, the relative humidity was on the average 7.1% lower than that of outside air; in isolators of our construction it was on the average 13.2% higher than that of outside air. It must be added that during the experiments the relative humidity of air inside the isolator never fell lower than 30%, whereas in control isolators such strong decreases in humidity took place rather often. The amelioration of the micro-climate thus obtained within the isolator had its influence on the seed yields. An average of the data obtained is given in Table 3.

TABLE 3

SUGAR-BEET SEED YIELDS AND WEIGHTS AS OBTAINED IN OPEN AIR AND IN ISOLATORS.

Type isolator	Yield per plant	Absolute weight of 1000 glomerules
None, flowering in open air	60.75 grams	16.7 grams
In isolator, cooled by water	59.07 "	14.1 "
In control isolators without cooling	38.08 "	11.4 "

d) Isolation of large area of plantings.

On the basis of experiments wherein we studied the distance to which the wind carried the pollen of sugar-beet in horizontal directions we may consider five to six kilometers between the planting areas as sufficient distance for practical purposes. Theoretically, of course, we cannot be sure that even with such isolation there will not be any hybrids. Hybridization is most likely to occur at the edges of plantations areas; in the central portions the pollen is more apt to be of one kind. It must further be noted that solution of the problem of distance isolation is not the same in all cases because many variables are involved. Of great importance are direction and strength of wind; weather during flowering—clear or rainy; the presence of different barriers for wind, such as trees; buildings; the activity of insects; and finally the size of the nearby plantings of sugar-beets.

MANIPULATION OF PLANT MATERIAL

a) Hybridization by artificial pollination after anther removal.

This method is described by Tschermak (44) who, on the basis of old data concerning protandry in sugar-beet, recommended Oliver's method in which the flower is rinsed out after the pollen sacs open. New data concerning time factors in fertilization of sugar-beet compelled us to reject this method. Instead it was necessary to castrate the flowers before they opened, then to place the desired pollen on the stigmas and lastly to bring the plant to seed under isolation. The question of the best time to apply the pollen arose, whether during castration operation or one or two days later. The greatest seed set occurred when the pollen was applied one or two days after castration. This operation, however, requires an extra opening of isolators. During the pollination operation it is quite possible for pollen from neighbouring sugar-beet plants that are in flower to reach the stigmas. Therefore, in order to minimize the percentage of outcrosses it is better to apply the chosen pollen at the moment of castration and after this to cover the plants with isolators (5).

In order further to reduce danger of contamination it is preferable to plant the roots in individual pots or other containers, and to carry out the operations of castration and artificial pollination in the laboratory or other protected place into which the pollen from other plants cannot penetrate. The plants are then covered with the isolator and returned to the field or greenhouse. The question often arises about preliminary preservation of pollen to make the desired matings. Very interesting observations concerning the conditions for preservation of sugar beet pollen have been carried out by Harechko-Savitzky (28).

b) Pollination between plant pairs under isolators without preliminary castration and without artificial pollination.

Munerati recommends (33) this method for obtaining pollinations between kindred plants and thereby furthering their inbreeding. Hjalmar-Nilsson (34) mated sugar-beets by placing the branches of neighbour plants within one parchment isolator. Frederiksen (19) recommends enclosing pairs of mother beets under cloth tents. Because of the fact that sugar-beets are frequently auto-sterile, fertilization between the plants takes place under the isolators. The convenience of the method lies in the fact that here the fertilization occurs automatically and along the entire stalk; as a result, a greater quantity of seed is obtained than by applying the methods of artificial pollination. In one of the writer's experiments the average percentage of hybrids between sugar and a red garden beet placed in pairs under an isolator reached 94.5%.

c) Hybridization in isolated seed plots.

The quantity of seeds obtainable from certain combinations of breeding stocks is very often insufficient if the experiment is restricted to methods *a* and *b* just described. If we wish to conduct an experiment in which F_1 progenies are compared (as, for example, in experiments with heterosis), relatively large quantities of seed are needed. In such case we must utilize a seed plot and depend on matings between free flowering plants. Here two possibilities are available:

I. Hybridization between individual plants of a given pedigree that are planted among a considerable number of plants of another kind.

Experiments of this sort have been carried out by Munerati (33), Sundelin (42) and Hallquist (26), and were repeated by the writer (3). Taking into consideration the practical auto-sterility of sugar beets, we have to consider that the percentage of hybrids should, in this case, approach 100%. But in each separate case subject to different conditions some deviations from this quantity are quite possible. For instance, in experiments performed by Hallquist (26) the percentages of hybrids in progenies varied from 73.6% to 100%; in experiments by the present author the hybrids varied from 70.2 to 96.66%. Such deviations can be attributed to general factors:

(*a*) differences in degree of auto-sterility of the individual plants;
(*b*) differences in the time of flowering of the two sorts being planted.
(c) effects arising from the ratio of plants of the one sort to plants of the other, or from pollen blowing in from nearby plantings of one of the varieties. According to Hallquist (26), the distance between plantings must not be less than 25 meters.

II. Hybridization between distinct sorts planted out in given proportions to one another.

The experiments of this type were carried out by Hallquist (26) and by the writer (53, 6). In the mating of two sorts (let us mark them A and B) the most convenient way is to plant the roots in the same numbers either in every other row or in checker-board pattern. It can then be assumed that seeds gathered from plantings A will give approximately 50% F_1 hybrids with B, and seeds gathered from B will give approximately 50% F_1 hybrids with A. However, from these theoretical percentages some deviations are quite possible for the following reasons: more or less divergence in time of flowering of components of the cross; differential quantities of pollen available from sorts A and B; differences in degree of compatibility of given sorts; and variation within sorts A and B with respect to the degrees of auto-sterility.

In one of the experiments conducted by the writer (5) the percentage of hybrids between the two sorts was 50.4. By changing the proportions between the sorts the percentage of hybrids in the progenies can be increased or decreased within the limits imposed by the factors listed.

LITERATURE CITED

1. ARCHIMOWITSCH, A. [Observations on the biology of flowering in the sugar beet] [Pl. Breed. & Seed-Grow. Adm. Bull.] No. 6: 94–102. 1923. [Russian; German summary.]
2. ————. [The problem of isolation of seed plants in sugar beet]. [Bull. Belaya Cerkov Pl. Breed. Sta. of the Sugar Trust] 1 (1): 7–36. 1927. [Russian; English summary.]
3. ————. [Regulation of the pollination in sugar beet]. [Bull. Belaya Cerkov Pl. Breed. Sta. of the Sugar Trust] 4 (2): 1–42. 1928. [Russian; English summary.] [See also U.S.S.R. Cong. Genet., Pl. & An. Breed. Proc.] 4: 29–40. 1930]. [Russian; German summary.]
4. ————. [Notes Belaya Cerkov Agr. Polytech. School.] 1 (1): 23–26. 1929. [Ukrainian.]
5. ————. [The problem of inbreeding in sugar beet.] [Bull. Belaya Cerkov Pl. Breed. Sta. of the Sugar Trust] 6 (3): 1–60. 1931. [Ukrainian; Russian summary.]
6. ————. Die Methodik der Isolation und Bastardierung bie der Zuckerrübe. Zeits. Züchtung A. Pflanzenzüchtung 15 (4): 357–365. 1930.
7. ———— *et al.* [Results of temperature and humidity study in the group isolators system of the Institute for Sugar Industry in Kiev]. [Scientific Notes on the Sugar Industry] 12 (4): 88–103. 1935. [Russian.]
8. ————. [A new type of isolator for groups of sugar beet plantings]. [Jour. Inst. Bot. Acad. Sci. RSS Ukraine] No. 26–27: 257–270. 1928. [Ukrainian; English summary.]
9. ————. [Causes of biological disparity in seeds from the same beetroot glomerule]. [Jour. Inst. Bot. Acad. Sci. RSS Ukraine. No. 31: 161–166. 1939.] [Ukrainian; English summary.]
10. ARTSCHWAGER, E. Development of flowers and seed in the sugar beet. Jour. Agr. Res. 34: 1–25. 1927.
11. ———— and R. C. STARRETT. The time factor in fertilization and embryo development in the sugar beet. Jour. Agr. Res. 47: 823–843. 1933.
12. BREWBAKER, H. E. Self-fertilization in sugar beets as influenced by type of isolator and other factors. Jour. Agr. Res. 48: 323–337. 1934.
13. BRIEM, H. Der praktische Rübenbauer. 534 pp. 1895.
14. BRIEM, H. Beitrag zur Kenntnis der Individualität des Rübensamens. Oesterr.-Ung. Zeit. Zuckerind. & Landw. 32: 907–912. 1903.

15. Darwin, Charles (Robert). The effects of cross and self fertilization in the vegetable kingdom. 487 pp. 1876.
16. Down, E. E. and C. A. Lavis. Studies on methods for control of pollination in sugar beets. Jour. Am. Soc. Agron. **22**: 1–9. 1930.
17. Favorsky, N. [Material for the biology and embryology of the sugar beet]. [Contr. Sci. Pl. Breed. Inst.] **2**: 1–18. 1928.
18. Fedorowitsch, L. J. [Some data applicable to use of inbreeding method in breeding sugar beets.] (Bull. Breed. Seed Imp., Sugar Trust) **4**: [Russian].
19. Frederiksen, E. [On the breeding of agricultural plants, especially with reference to the utilization of the diallel crossing method]. [Periodical for Land Economics] **1924**, (7): 357–403. 1924. [Danish].
20. Fruwirth, C. Die Züchtung der vier Hauptgetreidearten und der Zuckerrübe. Handb. Landwirts. Pflanzenzüchten 4th. ed. **4**: 449–455. 1923.
21. ————. Zur Frage der Selbstempfänglichkeit und der Inzestzüchtwirkung bei der Rübe. *In* Fests. zum 70. Geburtstage von Wilhelm Edler. 29–46.
22. German, I. V. [Extent of natural hybridization in sugar beet]. [Hybridization Information] No. 2, p. 102. 1941. [Russian].
23. Grinko, T. F. [On the method of hybridizing sugar beets]. [Bull. Exp. & Sel. Sta., Iwanowka] **4**: 47–63. 1927. [Russian.]
24. ————. [Self pollination in the sugar beet]. Proc. U.S.S.R. Congr. Genet. Pl. & An. Breed. **4**: 111–119. 1930. [Russian.]
25. ————. [Inbreeding in sugar beet]. [Beet culture] **1**: 727–741. 1940. [Russian].
26. Hallqvist, C. Ueber freiwilliges Selbstbestäuben bei *Beta*. Hereditas **9**: 411–418. 1927.
27. Harechko-Savitzky, H. [Flowering, fertilization and different types of sterility in *Beta vulgaris* L.] [U.S.S.R. Cong. Genet., Pl. & An. Breed. Proc.] **2**: 539–549. 1930. [Russian; English summary.]
28. ————. [Method and technique of artificial hybridization of the sugar beet]. [Works Sci. Res. Inst. Sugar Ind.] **13**: 613–632. 1931. [Russian; German summary.]
29. ————. Eine Methode der Samengewinnung bei Selbstbestäubung autosteriler Rübensorten. Compt. Rend. Acad. Sci. URSS, **18**: (7): 471–476. 1938.
30. Van Heel, Dudok, J. P. [Investigations on the development of the anthers, the ovule and the seed of *Beta vulgaris*.] Diss, Delft (Naarden) 1925.
31. Kohls, H. L. Seed production of space-isolated *vs.* bagged mother beets and a discussion of some factors influencing the latter. Jour. Am. Soc. Agron. **27**: 699–706. 1935.
32. Meier, Fred C. and E. Artschwager. Airplane collections of sugar-beet pollen. Science **88**: 507–508. 1938.
33. Munerati, O. [Observations and research on the sugar beet]. Atti Reale Accad. Lincei (5) Mem. Cl. Sci. Fis. Mat. e. Nat. **13**: 175–322. 1920. [Italian].
34. Nilsson, N. Hjalmar. [Practical beet breeding in Svalöf by new methods]. Sver. Utsädesf. Tidsk. **32**: 221–251 1922; **33**: 75–92. 1923.
35. Oksijuk, P. [Embryology of the sugar beet (*Beta vulgaris*)]. [Bull. Bot. Gard., Keiv.] **5–6**: 148–164, 1927. [Ukrainian; German summary].
36. ————. Zur Blütenbiologie der Zuckerrübe. Ber. Deut. Bot. Ges. **46**: 403–408. 1928.

37. ————. [On the biology of blossoming in the sugar beet]. Jour. Inst. Bot. Acad. Sci. RRS Ukraine] No. 9: 75–84. 1934. [Ukrainian; English summary.]

38. ORLOWSKY, N. T. [Beet field breeding]. Magazine Moscow No. 4, 1937. [Russian].

39. RALEIGH, S. M. Environomental factors affecting seed setting in sugar beets. Jour. Am. Soc. Agron. **28**: 35–51. 1936.

40. RIMPAU, W. Züchtung auf dem Gebiete der landwirtschaftlichen Kulturpflanzen. *In* Mentzel and Von Lengerke, Landw. Hülfs-und Schreib-kalender 1883, pt. 2, pp. 83–92. Berlin, 1883. (Die Runkelrübe, pp. 79–92.)

41. SHAW, H. B. Thrips as pollinators of beet flowers. U. S. Dept. Agr., Bull. 104. 1914.

42. SUNDELIN, G. [Contribution to biology of flowering of the genus *Beta*]. Sver. Utsädesf. Tidskr. **36**: 153–176. 1926.

43. TOWNSEND, C. O., and RITTUE, E. C. The development of single-germ beet seed. U. S. Dept. Agr., Pl. Ind. Bull. 73. 26 p. 1905.

44. TSCHERMAK, E. VON. BASTARDIERUNG. *In* C. Fruwirth. Handbuch der landwirtschaftlichen Pflanzenzüchtung. **4**: (Ed. 4.) 477–483. 1923.

45. UZEL, H. Ueber die Insekten, welche die Blüten der Zucker—und Futterrübe besuchen. Zeits. Zuckerind. Böhmen **37**: 182–197. 1913.

46. VILMORIN, J. L. de L'Hérédité chez la betterave cultivée. 153 p. 1923.

47. VOLKENS, G. Chenopodiacea. *In* Engler and Prantl Die natürlichen. Pflanzenfamilien III, I abt. A: 36–91. 1893.

48. WEGER, N. Über "Tütentemperaturen". Bioklimatische Beiblätter **5** (1): 16–19. 1938.

49. ZAJKOVSKAJA, N. E. [Comparative tests on autofertile varieties of sugar beet at the Glavsakhar selection stations]. [Sci. Notes Sugar Ind.]. **11**: (6): 18–32. 1934. [Russian].

50. ZOSSIMOWITSCH, V. P. [Weight and quality of sugar beet seeds in different blossoms of glomerules]. [Sci. Notes Sugar Ind.]. **12**: (5) 6: 128–131. 1935. [Russian].

51. ————. [Center of origin and history of the cultural beet.] Soviet Sugar **4**: 49–61. 1936.

SEVENTH INTERNATIONAL BOTANICAL CONGRESS,

STOCKHOLM 1950.

The Seventh International Botanical Congress will be held in Stockholm between July 12 and 20, 1950. Communication No. 3 from the Organizing Committee containing information on time of final application, membership fee, costs of excursions, expenses in Sweden, visa and money regulations etc. is now being distributed. It can be obtained from the Secretary General Dr. Ewert Åberg, Uppsala 7, Sweden.

THE NATURE OF THE FUNGICIDAL ACTION OF COPPER AND SULFUR[1]

S. E. A. McCALLAN

Boyce Thompson Institute for Plant Research, Inc., Yonkers 3, N. Y.

INTRODUCTION

The action of most copper and sulfur fungicides in the agricultural field, with which this paper will be primarily concerned, is protective in nature. That is, fungicidal sprays or dusts are applied, for example, to the healthy leaf or seed, in order to protect it from infection by fungus spores or mycelium. Such fungicides are necessarily relatively insoluble in order that they will not injure the foliage or be washed away by rain. Thus the question arises as to how these insoluble materials can exert their action and prevent the spores from germinating, possibly even killing them, so that the plant is protected from infection.

Despite the first use of a fungicide, namely sulfur, from the time of antiquity, and the practical significance of the copper fungicides dating from the discovery of Bordeaux mixture in 1882, there are still differences of opinion on the mechanism of action of these two most important groups of fungicides. With the increasing interest in improving our old fungicides and especially in developing new organic fungicides, it becomes of considerable practical importance to understand more fully the nature of fungicidal action, for by so doing we shall expedite considerably our search for new fungicides. A brief discussion of the prevailing views follows with particular emphasis on the more recent contributions.

COPPER FUNGICIDES

Copper fungicides may be grouped into three general types: basic salts, normal salts, organic complexes. By far the greater part of all research on copper fungicides has been on Bordeaux mixture, including attempts to explain the nature of fungicidal action. Bordeaux is prepared by mixing a solution of copper sulfate pentahydrate with a suspension of hydrated lime, one or both being in dilute form. The proportions of each may be varied, but usually the lime is equal to or in excess of the copper sulfate by weight. The chemistry of the resulting mixture is complex and has stimulated much research and many theories. At present the more recent work of Martin (32), which substantiates in part the earliest theories, seems to be generally accepted. Martin demonstrated that the blue precipitate of Bordeaux mixture is hydrated cupric oxide stabilized by adsorbed calcium sulfate.

---

[1] Presented at "Symposium on Chemicals for Agriculture", American Chemical Society, Washington, D. C., August 30, 1948.

Most investigators are agreed that for copper to be fungicidally active it must become soluble in water. While small amounts of soluble copper can be detected in freshly made Bordeaux, the amount found in air-dried films, as would happen in actual practice, is very small. From 0.2 to 0.3 p.p.m. (29) and 0.5 p.p.m. (48) have been found, amounts too small to materially affect the germination of most fungus spores. In order to explain how this practically insoluble copper of the copper fungicides, especially Bordeaux mixture, goes into solution, various theories have been advanced. Four in particular have received most attention. These are that (*a*) CO_2 and other "atmospheric agencies", (*b*) excretions by the plant or (*c*) secretions or exudates by the fungus are primarily responsible, while a fourth (*d*) holds that spores are able to absorb and cumulate copper from very dilute solutions and thus build up toxic amounts. Detailed discussions of the earlier work may be seen in a former paper (25).

CUMULATIVE ACTION. The cumulative action hypothesis rests largely on Nageli's work on oligodynamic action or poisoning by exceedingly small quantities of metallic substances in solution. Spores have been perfused with sub-toxic concentrations of copper (16) and cumulated enough copper to inhibit germination. Recent work (30) shows that spores of *Sclerotinia fructicola* are able to concentrate large amounts within themselves from very dilute solution. Martin (33, p. 138), however, concluded: "the small solubility product of the relatively insoluble copper compounds now known to be fungicidal does not encourage the acceptance of hypotheses based solely on solubility in water".

ATMOSPHERIC AGENCIES. The action of CO_2 and ammonia in rain water and dew was much favored by earlier workers, beginning with Millardet and Gayon (36). Bedford and Pickering (5) especially expounded this view and showed that copper was brought into solution by the action of CO_2 on Bordeaux. Gimingham (14), however, found that all of the soluble copper was reprecipitated when the CO_2 was withdrawn.

Goldsworthy and Green (15) have reported that Bordeaux deposits weathered out-of-doors gradually lost their toxicity to spores, even though copper was still present. In any event it is apparent that no special properties of rain or "meteoric water" are required for Bordeaux to exert its action, since the deposit is toxic in distilled water (25).

It has been observed (55) that weathered Bordeaux deposits changed in composition, becoming proportionately richer in copper, and that they also showed an increase in soluble copper. Carbonation of the excess lime was complete in a few hours, but the increases in soluble copper appeared much later. The amount of soluble copper, up to 0.2 mg. per 100 sq. cm., was considered sufficient to explain in part the fungicidal action of Bordeaux mixture as well as its phytotoxic action.

PLANT EXCRETIONS. The theory of plant or host excretions has received much speculation but relatively little actual investigation. As in the case of special "meteoric water", it obviously cannot be the sole factor, since Bordeaux exerts its toxic effect *in vitro,* and hence at best the action can be only supplementary. Recently Curtis (8) has shown that guttation fluid from various leaves is capable of bringing copper into solution from Bordeaux and other copper fungicides and also that such fluid increased the fungicidal action of sprayed films on glass. The hypothesis is advanced that guttation fluid increases the solubility of the pesticide which is then sucked back into the leaf, causing injury. It seems probable that guttation fluid and other leaf secretions would play an important role in phytotoxicity.

SPORE SECRETIONS OR EXUDATES. The action of spore secretions has appealed particularly to plant pathologists. This theory was first propounded by Swingle in 1896 (46) and has received the attention of various more recent workers (esp. 2, 25, 29, 48, 51). The early controversy between Barker and Gimingham (2) in favor of the role of the spore secretions *versus* the Duke of Bedford and Pickering in opposition is now historical. More recently it was shown (25) that the filtrate from distilled water in which spores had germinated, when added to a dried Bordeaux deposit and again filtered, brought enough copper into solution to be toxic to a second lot of spores. The check filtrates were without effect on spore germination. Later McCallan and Wilcoxon (29) found that water extracts from various species of fungi were active in rendering the copper of dried Bordeaux soluble and that in general the species excreting the greatest amount of solids were the most active in solubilizing copper and also were the most sensitive to the toxic action of Bordeaux. About three per cent of malic acid was identified in the solid matter obtained from the excretions of spores of *Neurospora sitophila,* and the presence of amino acids was also indicated. Glycine, aspartic acid and sodium malate dissolved large amounts of copper from Bordeaux mixture, while sodium cuprimalate and a copper glycine derivative exerted substantially the same toxic effect on fungus spores as copper sulfate. Thus it was postulated that the salts of hydroxy acids (malate and perhaps others) as well as of amino acids present in the spore excretions act on Bordeaux mixture to form soluble toxic copper salts of hydroxy and amino acids (29).

This line of attack has also been carried further by Wain and Wilkinson (48, 51). These workers confirmed the solubilizing action of *Neurospora sitophila* spore filtrates and found that temperature and nutrient medium were factors. The solvent properties of aqueous solutions of amino, hydroxy and other organic acids were shown (48). Mannitol, succinate and fumarate were isolated from spore exudates, but the solvent action of their aqueous solutions is relatively weak. However, malate was not isolated, possibly due to differences in medium (51). The hypothesis is presented that cupri-complexes formed by the action of the spore exudates on the copper fungicide dissociate, allowing the active toxicant to be removed by the spore. Studies by these workers (49, 50) on the effect of copper protectants applied to pea seeds as protection against soil fungi showed that colloidal protein-like material and certain amino derivatives were exuded from the seed and brought copper into solution which resulted in both fungicidal and phytotoxic action.

THE DOSAGE-RESPONSE CURVE. Considerable impetus has been given to the study of the nature of fungicidal action by the relatively new approach of examining the dosage-response curves. Gaddum (13) and Bliss (6) had shown that the individual lethal doses for a population of organisms are in many cases normally distributed against the logarithm of the dose, as shown in Figure 1. When the cumulated data are plotted, as is the case in practice, a symmetrical sigmoid curve results for percentage response on log dose (Fig. 2 A). If the percentage values are converted to units of the standard deviation, *i.e.*, normal equivalent deviations of Gaddum (13) or probability units or "probits" of Bliss (6), a straight line response will result. The slope of this curve is a measure of the uniformity of response of the individuals, greater uniformity being exhibited in greater steepness, and hence it gives some indication of the mode of action. In practice these curves are commonly plotted on logarithmic probability paper where no transformations are required (56), as illustrated in Figure 2 B.

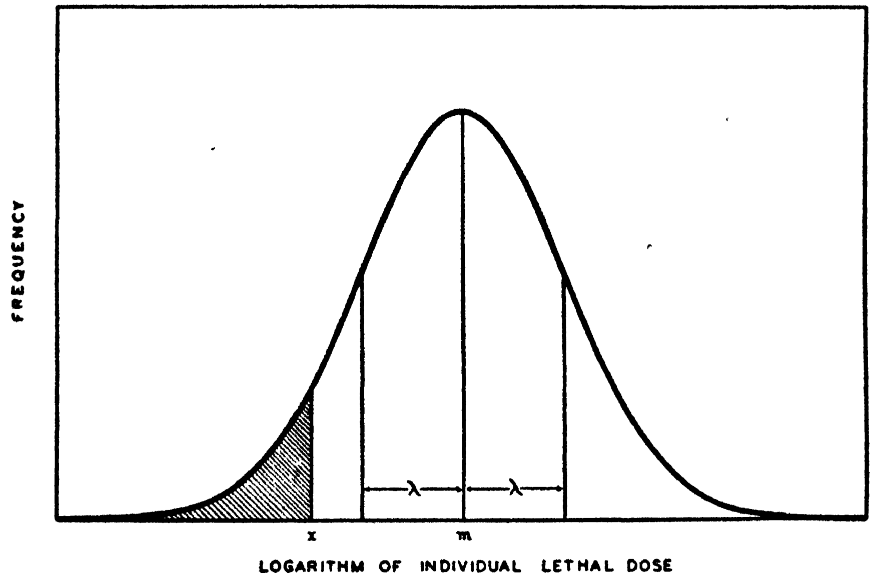


FIG. 1. Hypothetical distribution curve of individual lethal doses. Mean of individual lethal dose is *m* and standard deviation is λ. All spores having an individual lethal dose less than concentration *x* fail to germinate.

Dosage-response curves of fungicides have been studied (esp. 9, 28, 35, 38–43). It was found that the type and steepness of slope were determined more by the compound than by the fungus (28). Although the probit-log dose theory calls for a straight line response, non-linearity has been observed in a number of cases. The departures from linearity are of several types: (*a*) J curves, concave upwards, with the percentage death or spore inhibition greater than would be expected at low dosages; (*b*) the reverse of (*a*), with J curves convex upwards; (*c*) sigmoid curves, com-

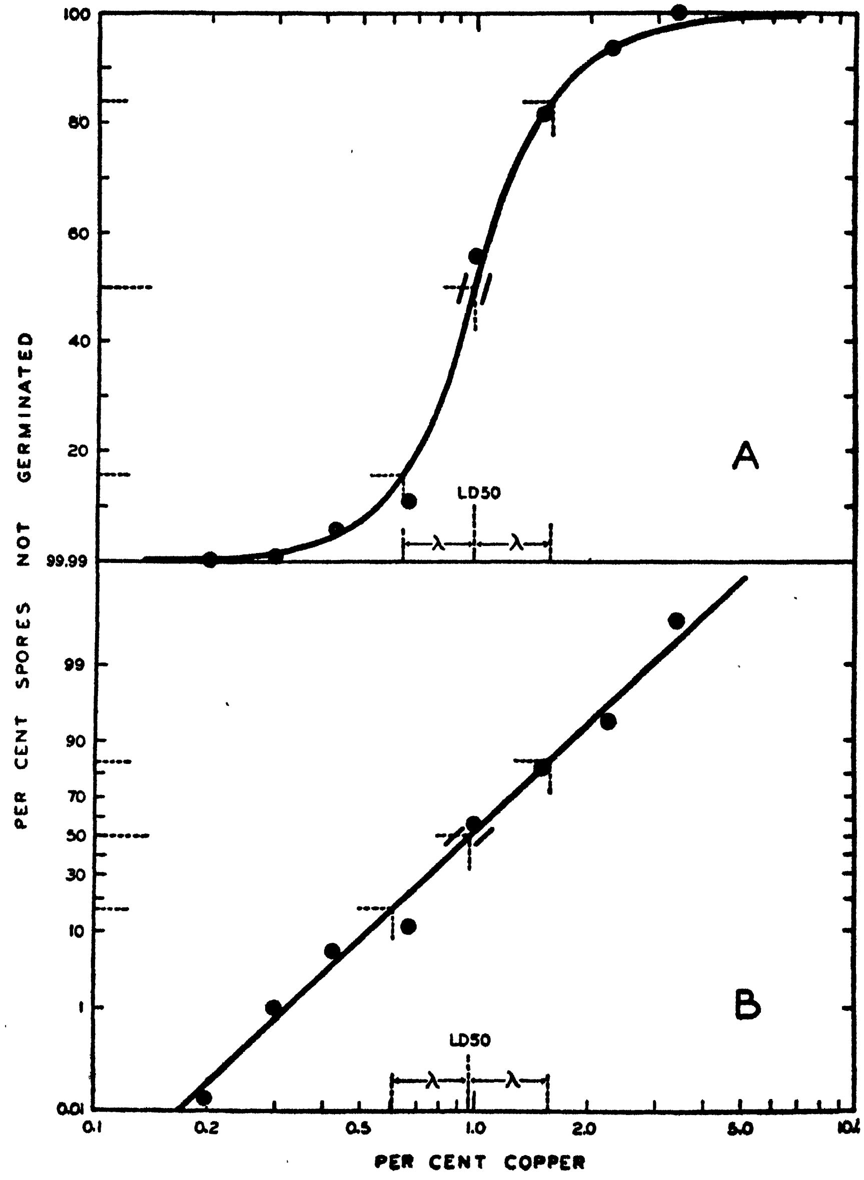


FIG. 2. Toxicity curves for action of a copper fungicide on spores of *Stemphyllium sarcinaeforme*. A. Per cent non-germination plotted against logarithm of concentration. B. Same data plotted on logarithmic probability paper.

bining (*a*) and (*b*) types; and (*d*) double maximum curves. The (*a*) type curves are the most common exceptions. Bliss (7) believed this was due to the action of two different toxic agents. Martin *et al.* (35) would further separate this type curve into two, depending on whether the "break" in the curve occurred at the low or high concentration. Their explanation for the former case, based on the Arndt-Shutz law that low concentrations of a toxicant act as a stimulant, would appear more fitting for the (*b*) type curve above. Other suggested explanations for the (*b*) type are that antagonism between the metal and hydrogen ions results in a changed distribution of resistance above a certain concentration (28) or that there is more than one toxic constituent (35). Types (*c*) and (*d*) do not appear to have been found with copper compounds.

Dosage-response curves for various inorganic and organic copper compounds have been examined (35). A large group of compounds, such as cupric chloride, phosphate and sulfate, yellow cuprous oxide and Bordeaux, exhibited a common regression coefficient, *i.e.*, slope which was attributed to the cupric ion. Basic derivatives had smaller coefficients. Derivatives in which coordination with the formation of complex ions can occur may exhibit more than one regression coefficient, a tendency correlated with stability of the complex ion. The hypothesis is advanced that in Bordeaux mixture and most relatively insoluble derivatives giving the cupric ion regression coefficient, there is solution by the spore exudate by complex ion formation, yielding cupric and complex ions of which the cuprimalate is that of most direct toxicity. Soluble derivatives yield cupric ions which interact with the spore exudate and proceed in the same manner. With yellow cuprous oxide there is a decomposition, so that half the copper reaches the spore by the cupric ion route. The process with various other compounds, such as cupric oxide and basic compounds, was too complicated to interpret.

THEORY OF VARIABILITY. Parker-Rhodes in a series of papers (38–43) has advanced a "theory of variability" to explain the mechanism of fungicidal action. It is postulated that the toxic action may take place in a series of steps and that the more steps the greater the variability of the spore population, since the final variability will be the sum of the variabilities of each spore to each different step. Thus the more steps the flatter the dosage-response curve. By a study of the slopes of various possible compounds of a given toxic element, *e.g.*, copper, it may be deduced that the compound with the steepest curve is the one that actually inhibits or kills the spores.

The spores are said to be normally distributed against the concentration raised to some power α, the index of variation. The value of α is determined by trial and error till the best straight line results. However, the basic concept is variability or U. $U = 1/\alpha^2 m^2 b^2$ where m is mean tolerance and b the slope. The statistical significance of U and α may be determined by appropriate formulae. The U values obtained for a series of compounds of a toxic element are expressed as simple ratios of one another, while the number of atoms of the effective elements in the molecule is the square of U and inverse of α. Thus three copper compounds containing one, two and three reactive atoms of Cu would be expected to give U values in the ratio of 1, 4 and 9, and α values of 1.0, 0.5 and 0.33. Variability is affected by number of atoms in molecule, number of steps to form a permeative compound and the value of the equilibrium constant K, since the reactions taking place are also postulated in terms of equilibrium constants.

In the case of copper it was inferred that "copper is absorbed by the spores more readily in the form of the monohydrated dithioureocuprous ion, or related compound, than as the simple cupric ion; certain cupric complex compounds tested appeared to require decomposition before absorption" (38, p. 404).

This is an ingenious hypothesis, and the use of the more versatile α exponential transformation to straighten dosage-response curves is an advantage over the commonly used logarithmic transformation and appears equally logical. It has been pointed out (26) that convex curves of the (*b*) type will require a negative exponential transformation. However, the weakness of the theory of variability is that the values for α often lie between simple numbers, the number of steps in the toxic reaction and the equilibrium constants are not known and must be postulated, and on hypothetical grounds a choice must be made as to which of a number of theoretically possible chemical compounds or reaction compounds is responsible for the toxic action.

As to how the copper kills, once it has permeated the spore, there is need for much more information. Lin (24), studying the effect of electrolytes on spore germination and on the toxicity of copper sulfate, found a marked antidotal effect of the electrolyte on poisoning by copper. This he explained as due to peptization of the precipitated colloids. Marsh (30) confirmed the antidotal effect of salts, and observed that it was associated with an increase in respiration and decrease in copper absorption, which he believed was due to ion antagonism. From studies on the injuriousness of copper fungicides applied to seeds, Foster (11) concluded that the copper destroys sulfhydryl groups essential for the respiratory system.

SULFUR FUNGICIDES

Because of its longer history, speculation on the mode of action of sulfur fungicides has been greater than with copper and has led to even more diverse opinions. Even fairly extensive investigation has not yet brought about complete accord. The advent of very promising new organic sulfur fungicides has increased the importance of and interest in a better understanding of the action of sulfur on fungi.

Inorganic sulfur fungicides, from a practical consideration, are of three main types: ground sulfur dust; modified sulfurs of varying kinds, including pastes and wettable sulfurs; and thirdly, lime sulfur. In addition in special cases sulfur may be applied by volatilization. Although lime sulfur which is prepared by boiling together sulfur and calcium hydroxide is a polysulfide in fresh liquid form, when diluted and sprayed on plants and dried, a series of changes in composition are initiated. The attempted explanation of these chemical changes has promoted more articles even than those to explain the chemistry of Bordeaux or any other spray material (12). It now seems more or less agreed however, that the end result of the decomposition changes is the precipitation of the polysulfide sulfur as finely divided free sulfur. Thus the protective fungicidal action of all the above forms of sulfur are in the end dependent on free elemental sulfur.

Since sulfur is insoluble in water, somewhat the same questions must be answered as in the case of insoluble copper, to explain its fungicidal action. "There is scarcely a compound of sulfur which might conceivably be formed from the element, under conditions of use, to which its toxic action has not been ascribed" (52, p. 391). Among these compounds are sulfur dioxide, sulfuric acid, thiosulfuric acid, pentathionic acid and hydrogen sulfide. Even the element itself in finely divided or vapor form has been suggested as the active agent. As pointed out before (52), "there is a great diversity of opinion expressed and much of the early work is inconclusive and not supported by adequate experimental evidence". There are extensive reviews of the earlier work (*e.g.*, 10, 18, 19, 45, 58).

ACTION AT A DISTANCE. Various investigators have shown that sulfur could act "at a distance". To explain this, some of them (3) postulated a "particulate" state, but this state was demonstrated by others (18, 19) to be merely sulfur vapor which would subsequently condense.

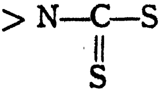
OXIDATION PRODUCTS. Earlier views that the action was due to sulfur dioxide or sulfuric acid do not seem to be generally substantiated. Young and associates in a series of papers (21–23, 57–59) have advanced the theory that pentathionic acid, an oxidation product of sulfur, is the active toxic agent. These conclusions were challenged (44, 52) on the grounds that the H-ion concentration of pentathionic and related acids could explain its toxicity, which is identical with sulfuric acid, and that the neutral salts were non-toxic. It was concluded that while traces of pentathionic acid may be present in sulfur fungicides, the acid is not a factor of importance in the fungicidal action (52).

HYDROGEN SULFIDE. One of the oldest and at present most actively supported theories is that hydrogen sulfide is responsible. This was first proposed by Pollacci in 1875. Marsh (31) and Barker (1) demonstrated that hydrogen sulfide was produced when various species of higher plants and fungi were dusted with sulfur. Marsh (31) also showed that hydrogen sulfide was toxic to germinating spores. These studies were carried further by McCallan and Wilcoxon (29) who found that the relative susceptibility of eight fungi to sulfur and to hydrogen sulfide was the same; also that if the production of hydrogen sulfide by these same spores was expressed in units equal to the amount necessary to reduce their germination 50 per cent, the sulfur-sensitive ones produced more than one unit and the sulfur-resistant ones less than one unit. Horsfall (20) showed further that if the spores of the sulfur-sensitive *Sclerotinia fructicola* are mixed with the spores of the sulfur-resistant *Macrosporium sarcinaeforme* in the presence of sulfur, the latter are killed or at least inhibited from germinating, presumably because of the excess H_2S produced by *Sclerotinia.*

It was previously found (52) that hydrogen sulfide was from six to 200 times as toxic as pentathionic or sulfuric acid to the spores of germinating fungi. However, against actively growing fungi it appears likely that hydrogen sulfide is somewhat less toxic (27, 34). McCallan and Wilcoxon (29) observed that actual contact between spores and sulfur was not necessary for reduction to hydrogen sulfide and that the reaction appeared enzymatic in nature, and the presence of an –SH group (possibly in glutathione) was detected in spores of *Sclerotinia.* The theory was advanced that sulfur vapor diffuses from the sulfur particles to the spores where it is reduced within or on the spores to form toxic hydrogen sulfide. Comparative studies with selenium and tellurium supported this theory (54).

PARTICLE SIZE. In addition to strictly chemical considerations, physical factors such as particle size have been shown to have an important bearing on fungicidal activity, especially with the sulfur fungicides. Thus it has been shown (53) that the toxicity of sulfur dusts increases with a decrease in particle size and that the dust furnishing the greatest number of particles per unit weight is the most toxic. Adherence of sulfur fungicides also has been found to increase with decreasing particle size (47, 53).

ORGANIC SULFUR FUNGICIDES. The most important organic sulfur fungicides and the only ones on which we have any appreciable information as to the nature of fungicidal action are the derivatives of dithiocarbamic acid and related compounds, the so-called dithiocarbamate fungicides. There are three groups of practical interest: the original tetramethylthiuram disulfide; metallic salts of dimethyldithiocarbamate, especially iron and zinc; and salts of ethylene bisdithiocarbamate, especially sodium and zinc.

In investigations (17) on the fungicidal properties of some metal dialkyl dithiocarbamates, the dimethyl compounds proved most fungicidal, the diethyl intermediate, and the dibutyl least fungicidal. The presence of the characteristic  $>N-\underset{\underset{S}{\|}}{C}-S$ group was not in itself sufficient to explain fungicidal properties. By a series of eliminating steps, Horsfall (20) argued that the toxicity of tetramethylthiuramdisulfide was probably due to the C=S rather than to the —S—S— grouping. However, many C=S compounds are relatively ineffective as fungicides. Parker-Rhodes (42) believed that the dithiocarbamates are decomposed by the spores into amine and carbon disulfide, both being toxic. These compounds, however, are very much less toxic than the dithiocarbamates.

One of the most noteworthy characteristics of this group of fungicides is the peculiar double maximum dosage-response curve exhibited by a number of the compounds. This type of curve (*d*) was recorded above in the discussion of dosage-response curves. This phenomenon was first reported by Dimond *et al.* (9) for tetramethylthiuram disulfide and independently confirmed for various thiuram sulfides, *etc.*, by Montgomery and Shaw (37). Dimond *et al.* (9) explained this on the basis of dissociation of the toxicant to a more toxic dissociated molecule. In an extensive study of the fungicidal action of the metallic alkyl bisdithiocarbamates, Barratt and Horsfall (4) observed this type of dosage-response curve also for sodium and ferric dimethyldithiocarbamate. It was "assumed that the peak at the high concentration is due to the liberation of H_2S by hydrolysis and rearrangement of the C=S group, and that the peak in the lower concentrations is due to the splitting of the molecule into two dithiocarbamate ions that could combine with metals in the spore to form direct salts" (4, p. 48). The general conclusion postulated on the basis of two known reactions was that disodium bisdithiocarbamate inhibits spores by one or both of two reactions, (*a*) by liberating H_2S which may alter the proteins of the spores by addition of sulfhydryl groups and (*b*) by forming metal salts by substitution of the sodium, that is, "the trace metals which the fungus needs in its metabolism are made unavailable either through the formation of metallic sulfides or metal salts of ethylenebisdithiocarbamic acid" (4, p. 48).

CONCLUSIONS

In summarizing available information on the mechanism of the fungicidal action of copper it appears most likely that exudates, such as hydroxy and amino acids, produced from fungus spores react with the "insoluble" copper fungicides to form soluble toxic copper complexes. These copper complexes exert the direct fungicidal action and vary in composition, depending on the original fungicide and probably also the fungus. While this is the primary fungicidal action, it is supplemented by the copper brought into solution by atmospheric agencies and host plant exudates. These two agencies, however, likely play the most important role in phytotoxicity or host plant injury. Finally cumulative action is also a supplementary factor, though perhaps indistinguishable from and a part of the action by spore secretions or exudates.

In the case of the sulfur fungicides the various theories of mechanism of action are more or less mutually exclusive. The evidence favors action at a distance by means of the vapor, and that the vapor can react with the spore or other plant tissue to produce hydrogen sulfide, the active toxic agent. Hydrogen sulfide produced by action of the plant is probably of importance only in phytotoxicity.

The dosage-response curve is a valuable tool in the interpretation of the nature of fungicidal action and should aid in a better understanding of this intricate mechanism.

LITERATURE CITED

1. Barker, B. T. P. Investigations on the fungicidal action of sulphur. IV. Third progress report. Long Ashton Agr. & Hort. Res. Sta., Ann. Rep. 1929: 130–148. 1929.
2. ————, and C. T. Gimingham. The fungicidal action of Bordeaux mixture. Jour. Agr. Sci. 4: 76–94. 1911.
3. ———— *et al.* Sulphur as a fungicide. Long Ashton Agr. & Hort. Res. Sta., Ann. Rep. 1919: 57–75. 1919.
4. Barratt, R. W., and J. G. Horsfall. Fungicidal action of metallic alkyl bisdithiocarbamates. Conn. Agr. Exp. Sta., Bull. 508. 1947.
5. Bedford, Duke of, and S. U. Pickering. Woburn Exp. Fruit Farm Rep. 11: 1–190. 1910.
6. Bliss, C. T. The calculation of the dosage-mortality curve. Ann. App. Biol. 22: 134–167. 1935.
7. ————. The toxicity of poisons applied jointly. Ann. App. Biol. 26: 585–615. 1939.
8. Curtis, L. C. The influence of guttation fluid on pesticides. Phytopath. 34: 196–205. 1944.
9. Dimond, A. E. *et al.* Role of the dosage-response curve in the evaluation of fungicides. Conn. Agr. Exp. Sta., Bull. 451: 635–667. 1941.
10. Doran, W. L. Laboratory studies of the toxicity of some sulphur fungicides. N. H. Agr. Exp. Sta., Tech. Bull. 19. 1922.
11. Foster, A. A. Acceleration and retardation of germination of some vegetable seeds resulting from treatment with copper fungicides. Phytopath. 37: 390–398. 1947.
12. Frear, D. E. H. Chemistry of insecticides and fungicides. 300 pp. 1942.
13. Gaddum, J. H. Reports on biological standards. III. Methods of biological assay depending on a quantal response. Privy Coun. Med. Res. Coun. Spec. Rept. Ser. 183: 1–46. 1933.
14. Gimingham, C. T. The action of carbon dioxide on Bordeaux mixture. Jour. Agr. Sci. 4: 69–75. 1911.
15. Goldsworthy, M. C., and E. L. Green. Availability of the copper of Bordeaux mixture residues and its absorption by the conidia of *Sclerotinia fructicola.* Jour. Agr. Res. 52: 517–533. 1936.
16. ————. Effect of low concentrations of copper on germination and growth of conidia of *Sclerotinia fructicola* and *Glomerella cingulata.* Jour. Agr. Res. 56: 489–505. 1938.

17. ——— *et al.* Fungicidal and phytocidal properties of some metal dialkyl dithiocarbamates. Jour. Agr. Res. **66**: 277–291. 1943.

18. Goodwin, W., and H. Martin. The action of sulphur as a fungicide and as an acaricide. Part I. Ann. App. Biol. **15**: 623–638. 1928.

19. ———. The action of sulphur as a fungicide and as an acaricide. Part II. Ann. App. Biol. **16**: 93–103. 1929.

20. Horsfall, J. G. Fungicides and their action. 239 pp. 1945.

21. Liming, O. N. The relation of pentathionic acid and its component constituents to the toxicity of sulphur fungicides. Phytopath. **22**: 143–164. 1932.

22. ———. The preparation and properties of pentathionic acid and its salts; its toxicity to fungi, bacteria and insects. Phytopath. **23**: 155–174. 1933.

23. ———, and H. C. Young. Toxicity of sulphur to spores of *Sclerotinia cinerea* as affected by the presence of pentathionic and other sulphur acids. Jour. Agr. Res. **40**: 951–962. 1930.

24. Lin, C. K. Germination of the conidia of *Sclerotinia fructicola*, with special reference to the toxicity of copper. N. Y. [Cornell] Agr. Exp. Sta., Mem. 233: 1–33. 1940.

25. McCallan, S. E. A. Studies on fungicides. III. The solvent action of spore excretions and other agencies on protective copper fungicides. N. Y. [Cornell] Agr. Exp. Sta., Mem. 128: 25–79. 1930.

26. ———. Characteristic curve for action of copper sulfate on the germination of spores of *Sclerotinia fructicola* and *Alternaria oleracea*. Cont. Boyce Thompson Inst. **15**: 77–90. 1948.

27. ———, and F. R. Weedon. Toxicity of ammonia, chlorine, hydrogen cyanide, hydrogen sulphide, and sulphur dioxide gases. II. Fungi and bacteria. Cont. Boyce Thompson Inst. **11**: 331–342. 1940.

28. ——— *et al.* An analysis of factors causing variation in spore germination tests of fungicides. III. Slope of toxicity curves, replicate tests, and fungi. Cont. Boyce Thompson Inst. **12**: 49–78. 1941.

29. ———, and F. Wilcoxon. The fungicidal action of sulphur. II. The production of hydrogen sulphide by sulphured leaves and spores and its toxicity to spores. Cont. Boyce Thompson Inst. **3**: 13–38. 1931.

30. Marsh, P. B. Salts as antidotes to copper in its toxicity to the conidia of *Sclerotinia fructicola*. Phytopath. **35**: 54–61. 1945.

31. Marsh, R. W. Investigations on the fungicidal action of sulphur. III. Studies on the toxicity of sulphuretted hydrogen and on the interaction of sulphur with fungi. Jour. Pom. & Hort. Sci. **7**: 237–250. 1929.

32. Martin, H. Studies upon the copper fungicides. I. The interaction of copper sulphate with calcium hydroxide. Ann. App. Biol. **19**: 98–120. 1932.

33. ———. The scientific principles of plant protection. 3rd ed. 385 pp. 1940.

34. ———, and E. S. Salmon. The fungicidal properties of certain spray-fluids. IX. The fungicidal properties of the products of hydrolysis of sulphur. Jour. Agr. Sci. **22**: 595–616. 1932.

35. ——— *et al.* Studies upon the copper fungicides. V. A critical examination of the fungicidal value of copper compounds. Ann. App. Biol. **29**: 412–438. 1942.

36. Millardet, A., et U. Gayon. Recherches nouvelles sur l'action que les preparations cuivrenses exercent sur le Peronospora de la vigne. Jour. d'Agr. Prat. **51**: 123–139, 156–161. 1887.

37. Montgomery, H. B. S., and H. Shaw. Behaviour of thiuram sulphides, etc., in spore germination tests. Nature **151**: 333. 1943.

38. Parker-Rhodes, A. F. Studies on the mechanism of fungicidal action. I. Preliminary investigation of nickel, copper, zinc, silver, and mercury. Ann. App. Biol. **28**: 389–405. 1941.

39. ————. Studies on the mechanism of fungicidal action. II. Elements of the theory of variability. Ann. App. Biol. **29**: 126–135. 1942.

40. ————. Studies on the mechanism of fungicidal action. III. Sulphur. Ann. App. Biol. **29**: 136–143. 1942.

41. ————. Studies on the mechanism of fungicidal action. IV. Mercury. Ann. App. Biol. **29**: 404–411. 1942.

42. ————. Studies on the mechanism of fungicidal action. V. Non metallic and sodium dithiocarbamic acid derivatives. Ann. App. Biol. **30**: 170–179. 1943.

43. ————. Studies on the mechanism of fungicidal action. VI. Water. Ann. App. Biol. **30**: 372–379. 1943.

44. Roach, W. A., and M. D. Glynne. The toxicity of certain sulphur compounds to *Synchytrium endobioticum*, the fungus causing wart disease of potatoes. Ann. App. Biol. **15**: 168–190. 1928.

45. Sempio, C. Sulla interpretazione del meccanismo intimo di azione dello solfo come anticrittogamico. Mem. della Reale Accad. d'Italia 3. Biol. No. 2: 1–30. 1932.

46. Swingle, W. T. Bordeaux mixture: its chemistry, physical properties, and toxic effects on fungi and algae. U. S. Dept. Agr., Div. Veg. Phys. Path. Bull. 9: 1–37. 1896.

47. Thatcher, R. W., and L. R. Streeter. The adherence to foliage of sulfur in fungicidal dusts and sprays. N. Y. [Geneva] Agr. Exp. Sta., Tech. Bull. 116: 1–18. 1925.

48. Wain, R. L., and E. H. Wilkinson. Studies upon the copper fungicides. VI. The solution of copper from Bordeaux and Burgundy mixtures. Ann. App. Biol. **30**: 379–391. 1943.

49. ————. Studies upon the copper fungicides. VII. The solution of copper from dressings on pea seeds. Ann. App. Biol. **32**: 240–243. 1945.

50. ————. Studies upon the copper fungicides. VIII. The penetration of copper into germinating peas. Ann. App. Biol. **32**: 243–247. 1945.

51. ————. Studies upon the copper fungicides. IX. Investigations with exudates from fungus spores. Ann. App. Biol. **33**: 401–405. 1946.

52. Wilcoxon, F., and S. E. A. McCallan. The fungicidal action of sulphur. I. The alleged rôle of pentathionic acid. Phytopath. **20**: 391–417. 1930. (*Also in* Contr. Boyce Thompson Inst. **2**: 389–415. 1930.)

53. ————. The fungicidal action of sulphur. III. Physical factors affecting the efficiency of dusts. Contr. Boyce Thompson Inst. **3**: 509–528. 1931.

54. ————. The fungicidal action of sulphur. IV. Comparative toxicity of sulphur, selenium, and tellurium. Contr. Boyce Thompson Inst. **4**: 415–424. 1932.

55. ————. The weathering of Bordeaux mixture. Contr. Boyce Thompson Inst. **9**: 149–159. 1938.

56. ————. Theoretical principles underlying laboratory toxicity tests of fungicides. Contr. Boyce Thompson Inst. **10**: 329–338. 1939.

57. Williams, R. C., and H. C. Young. The toxic property of sulfur, chemistry in relation to toxic factors. Ind. & Eng. Chem. **21**: 359–362. 1929.

58. Young, H. C. The toxic property of sulphur. Ann. Missouri Bot. Gard. **9**: 403–435. 1922.

59. ————, and R. C. Williams. Pentathionic acid, the fungicidal factor of sulphur. Science **67**: 19–20. 1928.

TRIARCH BOTANICAL PRODUCTS

Fine Microscope Slides for Critical Botanists

* * * * *

Nothing can take the place of a good microscope slide in botany instruction, especially to show clearly that structure is a basis for function. However, the slide must be *good,* even though it is not perfect. If the slide can be beautiful as well as clear, it becomes a fine piece of teaching equipment.

TRIARCH slides, we believe, embody these points more completely than any other slides on the market, as we stain them regularly with our famous quadruple combination of safranin, crystal violet, fast green and gold orange, unexcelled for tissue differentiation. Also, as we stain all sections by the same schedule, our slides give you comparative checks on age and development of tissues. Many cytological points are emphasized by Triarch stain, and host-parasite relations are clearly demonstrated in pathological tissues.

Increasing numbers of botanists are realizing the teaching value of Triarch slides, together with the convenience of Triarch accuracy and prompt service, as evidenced by the fact that since 1938 we have sold more than 500,000 slides to more than 1000 patrons in 19 different countries.

Our new 1949 Catalog, No. 8, is now being distributed. Write for a copy if you do not have one, and watch for special announcements in our quarterly publication, TRIARCH TOPICS.

* * * * *

Geo. H. Conant

Triarch Botanical Products :-: Ripon, Wisconsin

THE BOTANICAL REVIEW

VOL. XV DECEMBER, 1949 No. 10

NATURAL HYBRIDIZATION WITH PARTICULAR REFERENCE TO INTROGRESSION

CHARLES B. HEISER, JR.
Indiana University

DEFINITION AND CLASSIFICATION OF HYBRIDS

The word hybrid has come to have a very broad meaning, and a precise definition of it is difficult. Darlington (1937*a, b*) has defined a hybrid as "a zygote produced by the union of dissimilar gametes", and this definition if literally accepted means that practically all plants and animals are hybrids. Probably the majority of people think of hybrids as being derived from crosses between organisms belonging to different races, varieties or species. Darwin (1872) used hybrid exclusively to refer to the offspring of crosses between species, and spoke of the offspring of varietal crosses as "mongrels". There is much to be said in favor of the distinction made by Darwin, although of course there would always be cases difficult to place definitely in one category or the other. To say that the word hybrid is simply used by systematists as a label for misfits (Darlington, 1937*b*) can hardly be literally accepted today. The threefold nature of hybrids from the taxonomic, cytological and genetical standpoint has been considered by Dansereau (1940). The hybrids considered throughout the body of this paper, unless otherwise specified, will refer to hybrids between species or between entities designated as such by the particular investigator.

An attempt to classify hybrids also offers difficulties. Darlington (1937*a*) has classified them "by function" into the following seven groups: *a*) numerical hybrids; *b*) structural hybrids; *c*) undefined structural hybrids; *d*) complex hybrids; *e*) polyploid hybrids; *f*) numerical-structural hybrids; *g*) Mendelian hybrids. The polyploid hybrids form a distinct group which will not be considered here. Excellent reviews on the subject of polyploidy may be found elsewhere (Goodspeed and Bradley, 1942; Clausen, Keck and Hiesey, 1945; Stebbins, 1947*a*). The complex hybrids form a rather specialized class and appear to be somewhat rare outside the genus *Oenothera* (see Cleland, 1944). The Mendelian hybrids presumably would be hybrids within a species, but some of the hybrids discussed below might very well fall into this class. The remaining classes include a large number of the common natural hybrids. It is impractical at present, however, to apply Darlington's classification to hybrids in nature because so few natural hybrids have been analyzed cytogenetically. For convenience we may break down the natural hybrids into the following two groups: *a*) those which are completely sterile, the so-called "mules"; *b*) those which are not completely sterile and which by virtue of that fact may backcross to the parent species. The latter are the "recombination hybrids" of Anderson (1939*a*), and it is to this group that particular attention is to be devoted in the present review.

HISTORICAL

Focke in 1881 summarized the knowledge of that time regarding experimental and natural hybrids, and detailed accounts of the early history of hybridization may be found in Roberts (1929) and Zirkle (1935). Although naturalists have been aware of hybrids for more than 200 years, little intensive study of hybridization in nature was undertaken until recent times. With the rediscovery of Mendel's paper there was a reawakening of interest in hybridization, but for the most part the early studies following 1900 were devoted to the verification and extension of Mendel's law, using experimentally produced intraspecific hybrids. In 1916 appeared Lotsy's "Evolution by Means of Hybridization" in which he attempted to explain all evolution in terms of crossing. Although it received very little acceptance, this work did serve as a stimulus to further studies.

During the second decade of the twentieth century important studies on hybrids in the New Zealand flora were undertaken by Cockayne who was later joined by Allan (see Allan, 1931, for bibliography). Their work, for the most part, consisted of cataloguing the putative hybrids found in the wild, although in some instances actual crossing experiments were undertaken.

Natural hybridization between *Geum urbanum* and *G. rivale,* two species which show good morphological and ecological differences, has drawn the attention of a number of workers. The artificial cross between the two species has been made by Winge (1926) and others. In 1927 Ostenfeld (1928) delivered a lecture on hybridization in which he pointed out that species remain distinct in nature despite hybridization, referring particularly to the example of *Geum.* In spite of backcrossing of the hybrids to the parents, the two species remain distinct entities. The distinctness, he concludes, is maintained because the types nearest the parent species are best fit for existence and because the chances of the hybrid holding its own in competition with the parents are poor. Ostenfeld further pointed out that repeated backcrossing of hybrids to their parents may erase all external signs of hybridity. In 1930 Marsden-Jones produced experimental evidence to back many of Ostenfeld's statements concerning *Geum.* This latter study is particularly commendable in that detailed consideration is given to both the artificial and the natural hybrids. Marsden-Jones calls attention to the fact that backcrosses to *G. rivale* are almost indistinguishable from that species. At one locality in nature the backcross type appears to have become stabilized and largely to have supplanted one of the parent species. Marsden-Jones also records the appearance of a new character in some of the progeny of the artificial hybrids which was not found in either parent.

The work of Popov (1927) on the significance of hybridization in nature also deserves mention here, particularly since it has been generally overlooked by most students in this field. One example cited by him is of particular interest. *Zygophyllum Fabago* comprises two varieties, *Z. Fabago* var. *typicum,* predominantly a western race, and *Z. Fabago* var. *brachypterum,* an eastern race. Plants resembling *Z. Fabago* var. *typicum,* however, were found to occur in the eastern portion of the range of the species so that this variety apparently has a discontinuous distribution. A second species, *Z. obliquum,* occurs in the eastern part of the range, and Popov points out that hybridization between this latter species and *Z. Fabago* var. *brachypterum* could produce forms morphologically similar to *Z. Fabago* var. *typicum.* Hybridization thus explains the apparent disjunct distribution of this variety. Popov, however, produced no experimental evidence to back these claims.

This same period saw the initiation of studies on *Silene* by Marsden-Jones and Turrill (1928–1947), and interspecific hybridization was considered in the second paper of the series. The studies on *Geum,* on *Silene,* and those of Anderson and his collaborators on *Tradescantia* mark the beginning of careful attempts to analyze by experimental means the role of interspecific hybridization (without polyploidy) in nature. In the first of the *Tradescantia* papers (Anderson and Diehl, 1932) little space is devoted to hybridization. In the monograph of the American species of the genus (Anderson and Woodson, 1935) in which 15 putative hybrids are listed and seven artificially produced hybrids are discussed, it is pointed out that under certain conditions the germ plasm of one species might become "infected" through hybridization with a small amount of another species without any great morphological effect. Avenues for studies of hybridization are suggested in this paper, which have for the most part formed the foundation for future studies of natural hybridization. An attempt to analyze the effects of hybridization between certain species appeared shortly afterwards (Anderson, 1936*c*), and a method for measuring hybridization was introduced, which has enjoyed considerable popularity among subsequent workers. The second part of this study (Anderson, 1936*c*) is devoted to a consideration of natural hybridization between *T. canaliculata* and *T. virginiana.* Another detailed study appeared two years later (Anderson and Hubricht, 1938*a*) in which the term "introgressive hybridization" was introduced to refer to the transfer of germ plasm from one species to another by means of hybridization. The interaction of hybridization with the habitat was also discussed in this paper.

It is now, of course, apparent that the previously cited examples of hybridization in *Zygophyllum* and *Geum* involved introgression. In the following pages the subject of introgressive hybridization, or introgression as it is now generally termed, will be considered in some detail. A review of a large number of earlier examples of hybridization which are now seen to fall into this category may be found in DuRietz (1930) and Allan (1931, 1937, 1940, 1949). Clausen, Keck and Hiesey (1945) have also discussed a number of cases suggestive of introgression, although they are not labeled as such. In a recent textbook (Riley, 1948) a special section has been devoted to the phenomenon of introgression. The only previous review, however, treating introgression as such, is that of Cain in 1944; several additional studies dealing with introgression have been published since the appearance of this work which makes a comprehensive review desirable. It is still too early in many respects, however, to attempt a detailed critical analysis.

TECHNIQUES

There has been a great deal of skepticism among some biologists in regard to the great number of hybrids reported for plants, and it has been argued (Dobzhansky, 1941) that remnants of the ancestral population from which two species have differentiated might have the appearance of hybrids.[1] Secondly, it has been pointed out (Dobzhansky, 1941) that mutants arising within one species might display certain characters occurring in another species, giving rise to situations resembling hybridization. In view of this skepticism regarding hybrids, it seems desirable to consider the methods of recognition and study of natural hybrids.

Morphological. The oldest and still the basic starting point for recognition of hybrids is provided by a study of the morphology of the putative hybrid and its supposed parents. Some workers (Moss, 1930; Allan, 1937) have insisted that field observations alone can establish beyond doubt many groups of hybrids. It is, however, the large number of hybrids that have been reported on such circumstantial evidence and the non-statistical reporting of the data that have given rise to a large amount of the skepticism mentioned above.

Biometrical. By the use of population studies the older morphological comparisons have been placed upon a much sounder foundation. Through the use of "mass collections" (Anderson and Turrill, 1935; Anderson, 1941) or "population samples", as some prefer to call them (Woodson, 1947), frequency distributions can be made for the statistical study of certain characters. A method for the comparison of populations by means of "frequency histograms" or "hybrid indices" has been proposed (Anderson, 1936c; Hubbs and Kuronuma, 1941), which has received wide usage in the analysis of plant hybrids (Anderson and Turrill, 1938; Goodwin,

---

[1] An interesting example of such a case is afforded by Turrill's (1934) study of *Ajuga.* Plants intermediate between the species *A. chamaepitys* and *A. chia* occur in nature. Turrill interprets these intermediates as the common ancestors of the two species and not as hybrids.

1937*b*; Riley, 1938, 1939*c*; Wetmore & Delisle, 1939; Larisey, 1940; Dansereau, 1941; Dansereau & Lafond, 1941; Dansereau and Desmarais, 1947; Stebbins, Matske and Epling, 1947; Heiser, 1947, 1949). Baker (1947) criticises the use of such methods without adequate information governing the inheritance of characters used for scoring (see below). The polygonal graph method for population analysis (Davidson, 1947) might also prove useful in studying hybrid populations and in comparing the amount of genic contamination. The rigorous examination of population patterns by means of more detailed statistical methods offers a fertile field for studying the results of hybridization. Such an approach has been used by Woodson (1947) in analyzing variation in *Asclepias tuberosa.*

Ecological. A study of the habitats of the supposed parents and the putative hybrids is of fundamental importance in any analysis of the consequences of natural hybridization. The relationship of the habitat to hybridization has recently been emphasized (Anderson, 1948); a special section will be devoted below to a consideration of certain phases of the ecology of hybrids and their parents. The necessity for an understanding of the geographical distribution of the entities concerned should also be pointed out.

Genetical. To prove that hybridization can take place between two species, it is necessary that the actual cross be performed. In view of the apparent alarm at the "decline of experiment" (Baker, 1947) it is perhaps worthwhile to state the reasons why genetical experiments have not been more widely used in studies of natural hybridization. First of all, the taxonomist engaged in monographic studies frequently cannot undertake genetical studies. He should not neglect possible cases of hybridization, however, even though he is limited to morphological and field studies. Secondly, it is exceedingly difficult to germinate the seeds of many species, or to grow them under artificial conditions if germination is accomplished. In many genera, particularly woody ones, the length of time to flowering presents almost insurmountable difficulties for genetic experiments. It should be pointed out further that although two species may not cross under artificial conditions, this does not constitute absolute proof that they will not or have not crossed in nature. Nature carries on her experiments on a broad scale (Allan, 1937), and the investigator after a few attempts at artificial pollinations cannot say categorically that two species will not cross. However, plants that cross in nature and will not cross artificially are probably the exceptions.

The growing of an F_2 generation as well as the F_1 is, of course, to be recommended. A point overlooked by Baker (1947), however, is that if the F_1 is rather highly sterile, it may be nearly impossible to obtain an F_2 sufficiently large for genetical analysis. Although knowledge concerning the inheritance of characters in the particular cross is highly desirable, it must be remembered that the analysis of interspecific inheritance is not a simple matter, as is testified by the recent work on *Gossypium* genetics by Harland and others. Another point overlooked by Baker (1947) is the great importance of artificial backcrosses. It is principally by means of backcrosses that the germ plasm may be transferred from one species to another in nature. Particularly good examples of the use of artificial crosses, including both the F_2 generation and backcrosses, are provided by the studies of Goodwin (1937*b*) and Wetmore and Delisle (1939).

Progeny tests also may serve as a useful tool. The growing of seed from suspected hybrids, however, has been criticized (Baker, 1947) because no information is available as to the pollen parents. The fact that the pollen parent cannot be definitely established, however, does not rule out the usefulness of such an approach. The progeny study may often provide strong circumstantial evidence regarding the hybrid nature of certain individuals, and has been used to advantage in a number of cases (Anderson, 1936*a*; Wolf, 1938, 1944; Rollins, 1944; Heiser, 1947, 1949).

Cytological. In recent years information regarding the chromosome numbers and meiosis in the parents and putative hybrids has become very important in studies of hybridization. Irregularities in the meiosis of the putative hybrid have been used as additional evidence for the hybrid nature of the plants (Anderson and Woodson, 1935; Anderson and Sax, 1936; Riley, 1939*c*; McMinn, 1944; Heiser, 1947, 1949; Ross and Duncan, 1948). The cytological analysis of species hybrids has recently been reviewed in detail elsewhere (Stebbins, 1945) and need not be discussed here. A knowledge of the chromosome numbers, moreover, is of importance in separating amphidiploids from other types of hybrids (Camp and Gilly, 1943).

Information concerning relative fertilities may furnish a useful test of hybridity and may be obtained by very simple means in plants. The scoring of pollen grains as good (filled, normal sized grains) or bad (empty, undersized or misshapen grains) serves as an index to the male fertility and has been employed in a number of studies (Anderson and Woodson, 1935; Anderson, 1936*a*; Riley, 1939*b, c*; Larisey, 1940; Heiser, 1947, 1949). The use of various stains, acetocarmine, cotton blue in lacto-phenol, *etc.*, has now made such studies very simple, and they may be used on dried as well as fresh specimens. The relative number of good and bad seeds furnishes an indication of female sterility. In some cases the latter merely involves counting the number of filled and unfilled seeds or fruits, as in *Helianthus* (Heiser, 1947, 1949); in other cases, where the seeds are small, actual germination experiments have been undertaken (Goodwin, 1937*b*). It is, of course, realized that not all species hybrids are sterile and that sterility may result from causes other than hybridity. Darwin (1872) remarked on the fact that fertility may be influenced by the conditions under which the organisms are grown, and more recently it has been pointed out (Huskins, 1929) that bad pollen is not always the indication of a hybrid. Such routine studies as pollen and seed counts can be made fairly easily with most plants, even though more detailed cyto-genetic experiments may be out of the question, and no hybrid should be described without some reference to its fertility.

The desirability of special herbaria in connection with such studies and the importance of herbarium records of the material, including both natural and artificial hybrids, has been pointed out (Marsden-Jones, Summerhayes and Turrill, 1930; Turrill, 1938). Unfortunately, many workers do not state where the herbarium material has been deposited.

DEFINITION AND TYPES OF INTROGRESSION

The following account of introgression has been condensed from the original description of Anderson and Hubricht (1938*a*): Apparently the commonest result of hybridization is that through repeated backcrossing of the hybrids to the parental forms there is an infiltration of the germ plasm of one species into that of another. If two species capable of hybridizing meet, they usually do so in a situation more favorable to one than to the other. If hybrids are produced, they tend to cross back to the more abundant species. The progeny of these secondary hybrids are likewise crossed back again, and so on. Introgression then may be defined as the transfer of genes from one species to another by means of hybridization and backcrossing. The "infection" or "contamination" of populations previously reported by these and other authors refers to the same phenomenon.

If the two species hybridize and if the F_1 progeny is not completely sterile, it is possible for backcrossing to take place. There can be little doubt that such a process occurs in nature and that genes may be transferred from one species to another by such a method. The work of Hiorth (1933) on *Collinsia* and the work of the modern plant breeder have shown that genes can be so transferred by artificial hybridization. Harland (1936) states that genes can be transferred from one species to another by repeated backcrossing, even if the initial sterility of the F_1 is very great.

It is not yet possible to classify the types of introgression. However, we may attempt to define two rather broad classes as follows: *a*) the apparent gene flow may extend some distance from the area of the actual hybridization and spread to other populations (apparently this occurs in *Cistus, Hevea, Helianthus, Tradescantia, Vaccinium* and *Zea* (see Table 1 for references); or *b*) the apparent gene flow may be purely local and the effects of hybridization will not be noted away from the area of active hybridization (apparently in *Quercus, Salvia* and *Silene*—see Table 1). The gene flow that takes place may be chiefly in one direction, as, for example, of *Tradescantia canaliculata* into *T. occidentalis* (Anderson and Hubricht, 1938*a*); or it may proceed toward both species, as in *Parthenium,* which has been termed "reciprocal introgression" (Rollins, 1945*a*). Most of the examples cited in Table 1 have been too little analyzed to justify any attempt to place them definitely in either category *a* or *b* above.

In the genus *Balsamorhiza* (Ownbey and Weber, 1943) several different types of putative hybrids occur. In some instances the hybrids are "non-segregating and local", possibly an illustration of "mules"; in others they are "segregating and local", illustrating introgression of type *b*; in still others the segregating hybrids may have a wide range, illustrating introgression of type *a*.

The restricted or localized gene flow of type *b* above has rarely been called "introgression". However, it is clear that if backcrossing occurs there is usually contamination in the area of contact of the species. It is known that continual backcrossing may almost completely obliterate external signs of hybridity (Ostenfeld, 1928; Hubbs, 1940; Anderson, 1948), experimental verification for which goes back to Koelreuter (1893). The most conspicuous signs of hybridity are always in the region of active hybridization, and, although it may appear that there are no traces of the other species outside the zone of hybridization, it is still possible that more extensive gene flow has occurred than meets the eye. For example, if only a few genes pass from one species to another, the visible effects may be so slight as to easily escape detection. It requires exceedingly critical data to demonstrate the actual extent of introgression (Anderson, 1948).

INTROGRESSION IN HIGHER PLANTS

A selected list of examples of natural hybridization is presented in Table 1. The list is not intended to be exhaustive, but attempts to cover the majority of the work of recent years, particularly those cases which have been carefully analyzed. Not all of the examples cited have been labeled as introgression by the investigators, but it is apparent from their data or remarks that backcrossing takes place, so that theoretically at least there may be a gene flow between the species concerned. It is quite probable that many or all of the "multiform hybrids" described for the New Zealand flora (Cockayne and Allan, 1934) also fall into the province of introgression; the majority of these hybrids, however, have not been subjected to population analysis and are not included in the table. Although the evidence for introgression presented in several of the studies listed in Table 1 is far from conclusive, there can now be little doubt that such a process does occur in nature. All the examples given in the table are found either in the gymnosperms or among angiosperms. Although many hybrids are known for the pteridophytes, introgression has not yet been established for any of them. Benedict (1945) has recently called attention to the "problem and objectives in the study of fern hybrids", but he fails to mention the necessity for detailed population analyses.

From a survey of the works given in the table, a few general remarks may be made. First, it is apparent that the great majority of examples involve homoploid species, *i.e.*, species with the same chromosome number. However, it is also apparent that it is possible for introgression to occur between species with different chromosome numbers, as, for example, between diploid and tetraploid species (*Tradescantia*). It would appear, however, that differences in chromosome numbers provide a strong barrier to the occurrence of introgression. A second fact that is manifest from examination of the table is the great number of definitely established cases reported for the Compositae. Numerous hybrids are also listed for this family by Cockayne and Allan (1934). The large number of hybrids reported for the Compositae might be explained simply as the result of its large size or that by accident the hybrids in this family received more study. However, neither of these suggestions supplies an entirely satisfactory answer. It has been pointed out previously (Clausen, Keck and Hiesey, 1945) that the families Gramineae and Compositae, admittedly characterized by complex relationships, contain large numbers of polyploids. The amount of polyploidy, however, is considerably greater in the former family (Heiser and Whitaker, 1948). It might conceivably be that many of the complex relationships and taxonomic difficulties of the Compositae are in a large measure due to the amount of introgression as well as to the amount of polyploidy present.

A rather unusual case of gene flow between species by means of hybridization is found in *Galium* (not given in the table). The species *G. Mollugo* and *G. vernum* have both diploid and tetraploid races (Fagerlind, 1937). The tetraploids hybridize freely and give rise to a "delayed amphiploid" (Clausen, Keck and Hiesey, 1945); the offspring of the hybrid segregate, and by backcrossing to the parental tetraploid an exchange of genes between the species may be effected. In the delayed amphiploid of *Zauschneria californica* (Clausen, Keck and Hiesey, 1945) apparently there is preferential pairing of the chromosomes from the two parents and hence no opportunity for a gene bridge.

All cases of hybridization given in Table 1 concern entities which have been considered species by the particular investigators. It is not the intention here to go into the relative merits of regarding all of these as species; some discussion of this point will be entertained below. Hybridization on a subspecies level in plants has been analyzed in *Asclepias tuberosa* (Woodson, 1947). The three subspecies of *A. tuberosa* were originally isolated but are panmictic today. Measurements made from herbarium specimens and from population samples were treated statistically and then mapped by means of phenocontours. "Crests of variability", apparently representing heterosis, were found where the subspecies come together. In passing it should be noted that Woodson has presented evidence in this study which indicates that the herbarium offers a fertile field for statistical studies of this nature.

HYBRIDIZATION IN ANIMALS

It is well known that hybrids are much rarer in animals than in plants. Whether this is due to faulty data or to a greater efficiency of isolation between fully formed animal species is a matter that can be determined only by further studies (Dobzhansky, 1941). Mayr (1942) is inclined to think that "mistakes" leading to hybridization are much more common in plants than in animals, since fertilization is more or less a "mechanical process" in the former group. It could be, of course, that zoologists and botanists have tended to use different criteria in their delimitation of species and that in reality there is little difference in the amount of hybridization in the two groups. Backcrossing of animal hybrids to their parents, however, is known to occur in some instances, and the visible gene flow generally seems to be confined to the region of the hybrid swarm. Introgression on the whole appears to be extremely rare in animals.

No complete survey of the literature dealing with animal hybrids has been attempted, but a few examples seem desirable for inclusion here. Hybrid swarms are common in the moth genus, *Platysamia* (Sweadner, 1937), and, although the female hybrids are sterile, the males are partially fertile. Thus there can be an introgression through backcrossing of the males to the female parent strains. Sweadner comes to the conclusion that it is impossible to divide this group into species, sub-species or varieties. Hybridization is known to occur between certain species of toads (*Bufo*), and it appears that there may be some gene flow between species (Blair, 1941*a*, 1942). A possible instance of intergeneric hybridization between two tree toads has also been reported (Blair, 1941*b*).

Hybridization between species and even genera of fish in nature is now well established. Hubbs and co-workers (Hubbs, 1940;

Hubbs & Kuronuma, 1941; Hubbs and Miller, 1942; Hubbs, Hubbs and Johnson, 1943; Hubbs, Walker and Johnson, 1943; Hubbs and Hubbs, 1945; and additional references cited in these) have analyzed a number of cases in considerable detail. Backcrossing apparently takes place only rarely, as, for example, between *Siphateles* x *Gilia* (Hubbs and Miller, 1942). Natural hybrids occur between the muskellunge and northern pike, and the hybrids are at least partially fertile (Black and Williamson, 1946). Artificial backcrosses have been produced, but there is no mention of backcrossing occurring in nature. Introgression labeled as such has been described for fishes by Gordon (1946); however, in the example involved (*Platypoecilus* x *Xiphophorus*) hybridization is not known to occur in the wild, but has come about as a result of fish cultural practices. These species hybrids have been carefully analyzed genetically, and Gordon (1946*a, b,* 1947) reports that the W chromosome in domesticated stocks of *Platypoecilus maculatus* may represent a *Xiphophorus helleri* sex chromosome, the transfer having come about as a result of introgression.

Hybrids in the dog family have been discussed by Cole and Shackelford (1946). All of the hybrids between species of fox are unfertile, but hybrids between the dog and the wolf and possibly between the coyote and the dog are fertile. It is quite probable that considerable introgression occurs between the dog and wolf in many regions.

Numerous hybrids are also known for birds, and an excellent discussion of many of these may be found in Mayr (1942). Although Mayr states (p. 74) that introgression is "a theoretical process which in animals, at least, is not well supported by facts", some of the cases reviewed by him (in *Colaptes, Gennaeus, Passer, Junco,* pp. 263–270) are strongly suggestive of introgression to the present reviewer.[2] Attention also should be called to the similarity of introgression to the "secondary intergradation" of Mayr (1942). Both terms have been used to describe situations in which two entities, which presumably have differentiated in isolation, come together again to hybridize.

---

[2] Since the above was written, introgression has been described for the paradise flycatchers of Africa (Chapin, 1948).

NOMENCLATURE OF PLANT HYBRIDS

The naming of hybrids is a practical problem of grave concern to the taxonomist. The present provisions of the Rules of Nomenclature have come down only little changed since the last century (Allan, 1939), and, although liberalized, are now out of date in many respects. It has been stated that "the codes of nomenclature are nowhere more ineffectual than in their treatment of hybrids" (Anderson and Woodson, 1935).

Taxonomists have designated hybrids by the use of distinct names, usually prefixed by an X; by formula treatment; or by assigning them subspecific status. The so-called intergeneric hybrids are usually given a name compounded from the respective genera that have participated in the production of the hybrid; thus *Asplensoris* from *Asplenium* x *Camptosorus*. Friesner (1943) uses distinct names for two perennial *Solidago* hybrids, stating that it is much better to use a name for an entity which seems able to perpetuate itself indefinitely (by vegetative means presumably, although he does not state this), rather than the more cumbersome formula method. He has called these entities "sp. nov.", although the majority of writers prefer to use "hybr. nov." which seems more justifiable. Formulae have been used for hybrids in the annual species of *Helianthus* (Heiser, 1947, 1948) which are sporadic in their occurrence and which are not able to propagate themselves asexually.

Cain (1944) wonders whether the hybrids described by the taxonomist represent occasional F_1 plants, clones of F_1 plants or whether the taxonomist has attempted to draw a description to fit a few of the possible F_2's and backcrosses. Anderson and Hubricht (1938*a*) have stated that the application of "hybrid" and "species" is difficult in problems involving hybridization and backcrossing; the F_1 is clearly entitled to the name hybrid, but what about the backcrosses? It has been pointed out that it is the backcrosses which puzzle the taxonomist (Wetmore and Delisle, 1939).

If all hybrids were sterile and if introgression did not occur, the matter of nomenclature would be relatively simple. Certainly no hard and fast rules can be laid down regarding the naming of introgressants. In *Tradescantia* the effects of introgression appear to be too slight to merit nomenclatorial recognition (Anderson and Hubricht, 1938*a*), but in other cases it may be desirable to name the hybrid populations or derivatives. Dansereau (1941) and

Fassett (1945) have used the more or less noncommittal variety for this purpose in *Cistus* and in *Juniperus*. Camp (1941) has designated what appears to be a hybrid swarm by the name × *Befaria discolor* as a temporary nomenclatorial expedient. The use of clines for intergrading cases perhaps offers as satisfactory a method as any for the treatment of the hybrid derivatives among subspecies. The latter method has been used in the treatment of hybridizing subspecies of *Asclepias* (Woodson, 1944).

The question of the nomenclature of hybrids has received the attention of Allan, Sprague and Uittien in a special symposium. Uittien (1939) suggests that hybrids be completely divorced from taxonomy, for, according to him, parentage has no place in formal taxonomy. Some of Uittien's proposals have been criticized elsewhere (Fosberg, 1939, 1941; Heiser, 1949). Allan (1939) points out certain defects in the current schemes of classifying hybrids and suggests that each author must evolve a method best suited to his needs. His concluding remark that more studies of wild hybridism are necessary before its taxonomic importance can be assessed bears repetition here. Sprague (1939) replies to certain of Allan's criticisms of the rules and makes specific suggestions for the rewording of certain minor statements in the rules.

Echoing the sentiments of Allan, Camp and Gilly (1943) have stated that the fewer the nomenclatorial rules in regard to hybrids, the better. They write: "Because of their complicated nature, the naming of hybrids and populations derived from hybrids cannot be governed by a set of rules, unless the rules become so complicated that they defeat their own purpose". They go on to state that one cannot formulate rules to fit all cases and that the literature should not be encumbered by a series of useless names. It might be added here that it has yet to be shown that it is necessary or even desirable to name all hybrids or hybrid populations. The naming of hybrids has also been briefly discussed by Wiegand (1935) and Cugnac (1940), and recently in more detail by Rickett and Camp (1948) and Allan (1949).

From the foregoing account it can readily be seen that hybridization offers serious problems to the taxonomist. However, as has been pointed out previously (Heiser, 1947), an understanding of hybridization, particularly of the mechanism and effects of introgression, will often aid the taxonomist in the solution of what appears to be a most perplexing problem. One example will suffice to show the usefulness of such knowledge. In 1931 two reputable taxonomists (Small and Alexander, 1930) described over 80 new species of *Iris* from the Gulf States. It has since been shown from morphological, genetical and cytological evidence (Viosca, 1935; Foster, 1937; Riley, 1938, 1939*a, b*) that the great majority of these new "species" are simply hybrid derivatives resulting from the natural crossing of *Iris fulva* and *I. hexagona* var. *giganticaerulea,* and that it is extremely doubtful that any of them deserves specific recognition. This is a rather extreme case, but other examples are not wanting.

ECOLOGY OF HYBRIDS

Where hybridization has been the subject of special analysis, it has been found that the two parent species nearly always occupy different ecological niches[3]. It is also apparent in the great majority of cases that a disturbance of the environment has allowed the two species to come together. Man is frequently responsible for the latter by upsetting the normal ecological balance through such practices as lumbering, pasturing, road building and cultivation of the land. Brainerd (1906) associated the hybridization of the eastern species of *Viola* with the removal of forests by man; Cockayne (1939) has pointed out that hybrids may occur in great numbers following forest fires; and Wiegand (1935) has commented at some length on the relationship of man to hybridization, particularly in regard to the genus *Amelanchier.* The possible role of primitive man in creating opportunities for hybridization has also been commented upon (Sauer, 1947). Many others have pointed out the importance of man in providing opportunities for hybridization in plants (Turrill, 1929; Cockayne and Allan, 1934; Viosca, 1935; Anderson, 1936*c,* 1948; Anderson and Hubricht, 1938*a*; Goodwin, 1937*a*; Brett, 1938; Riley, 1939*c*; Camp, 1942; Black, 1944; Weber, 1946; Epling, 1947*b*; Heiser, 1947, 1949;

---

[3] One of the earliest ecological references to hybrids is found in the work of Hartmann, 1751 (see Zirkle, 1935, p. 172): "It is not strange that a hybrid which is the child of a swamp and a mountain plant should seek a third kind of soil. For this reason the grandfathers and great grandfathers are sought in vain next to the daughter plant". Attention should also be directed to the very early statement of Clausen (1922) that species remain pure in nature, since each has its own optimum type of soil and therefore two species are "only able to cross on the boundary lines of their respective areas of distribution".

Pettersson, 1947; Seibert, 1947, 1948; Woodson, 1947; Allan, 1949). Stebbins (1947*b*) has stated that a fourth great burst of evolution now beginning is due to alterations in the natural plant cover by man, and that introgression is playing a role. That hybridization in animals is frequently due to man's interference has also been clearly demonstrated (Blair, 1941*a*; Hubbs and Miller, 1942; Mayr, 1942; Gordon, 1946).

Man created not only opportunities for hybridization by bringing species together, but of equal importance he has also created intermediate types of habitats which are necessary for the success of the hybrid and its derivatives. Hubricht and Anderson (1941) have concluded that the lack of intermediate habitats between *Tradescantia subaspera* and *T. canaliculata* in the Ozarks prevents hybridization of these species in this region, since these species hybridize freely in the experimental garden and are known to hybridize in the Appalachians (Showalter, 1938). Anderson (1948) has pointed out the necessity of intermediate types of habitats for the survival of any hybrids or backcrosses beyond the F_1, and has emphasized that diverse intermediate habitats are required for the variously intermediate hybrid derivatives.

The importance of man in changing the environment should not obscure the possible role of natural forces and the occurrence of introgression before the appearance of man (Anderson, 1948; Heiser, 1949). Younger geologic areas, such as active growing deltas (Riley, 1939*c*), have been suggested as one such region where hybridization might occur. Glaciation may also have served as such an agent (Blair, 1941*a*; Camp, 1942). Man is not considered to have played the only role in producing areas for hybridization in *Salvia* (Epling, 1947*b*).

Environmental conditions may play an important role in the direction of introgression, and such is held to be the case in *Cistus* (Dansereau, 1943) and *Acer* (Dansereau and Lafond, 1941; Dansereau and Desmarais, 1947). In the latter case introgression is from *A. nigrum* into *A. saccharophorum* in the Laurentian range of the species "because the latter species is better adapted to the climate of southern Quebec". "The species less favored by present conditions will gradually be absorbed by the other, and the external characteristics of the hybrids will be more those of the preadapted species" (Dansereau and Desmarais, 1947). In view of the fact that no crosses have been made between these *Acer* species and that the evidence for hybridity rests upon morphological examination of only two characters, the case for hybridity can be considered only tentatively established. In a preliminary analysis of these two entities in other parts of their range, the conclusion was reached that there were strong barriers to their interbreeding (Anderson and Hubricht, 1938*b*).

Helianthus presents a slightly different aspect of the influence of environmental conditions. *Helianthus annuus* appears to have made a successful invasion of California by "absorbing" genes from a species (*H. Bolanderi*) already adapted to that climate; thus introgression has been into *H. annuus.* The gene flow has also proceeded in the reverse direction, from *H. annuus* into *H. Bolanderi,* with the creation of a distinct weedy ecotype of the latter species adapted to valley conditions (Heiser, 1949).

From the facts and theories accumulated from the literature presented in this section, certain generalizations can be made. First, species do not hybridize unless the normal ecological balance is disturbed in some way. If the disturbance is great enough, new types of environment may be created. If the hybrids are not sterile, backcrossing may take place and the hybrid derivatives become established in the new environments. The amount of backcrossing and consequently the amount of introgression is nearly always directly proportional to the "weediness of the environment" (Anderson and Hubricht, 1938*a*). Through such processes distinct races of the species may be created as in *Cistus* (Dansereau, 1941), *Juniperus* (Fassett, 1944, 1945), *Hevea* (Baldwin, 1947; Seibert, 1947) and *Helianthus* (Heiser, 1949) which are specially adapted to the new habitats. The importance of introgression to the origin of plants specially adapted to disturbed habitats then becomes obvious, and a possible role of introgression in the formation of weeds has been suggested (Anderson, 1948; Heiser, 1949).

BEARING OF NATURAL HYBRIDIZATION ON THE SPECIES PROBLEM

> "No one definition has satisfied all naturalists; yet every naturalist knows vaguely what he means when he speaks of a species".—C. Darwin (1872).

There is no need to undertake here an extended historical account of species definitions. For over 100 years biologists have attempted to apply or to reject the criterion of sterility in species definitions. Most biologists are inclined to agree, in spite of the failure of definitions, that species do exist in nature. In recent years, more so than ever before, it has been realized that species may differ in different groups of organisms. An attempt to define some of the different types of plant species has recently been made (Camp and Gilly, 1943).

The particular concern here is the effect of natural hybridization upon such definitions and upon the problem of the "species" in nature. As Anderson (1936*b*) has stated, hybridization may produce a slight blurring between two species, but few have felt that it could actually obliterate species differences.

First, are the entities considered here which meet and hybridize in nature entitled to specific rank? They violate one of the fundamentals of many definitions of a species. An essential of a species, according to Babcock (1931), is the absence of free intercrossing and usually low fertility if not complete sterility between different species. Elsewhere in the same paper he remarks, however, that it is now generally accepted that many true species will hybridize and that they will often produce partially fertile offspring, although in general the trend is toward sterility. (Babcock, as he himself points out, proposes no definition.) More recently, Dobzhansky (1941) states that hybridization on the boundaries of the distribution areas between species does not invalidate the general concept.

There is also the paradox of the species of the taxonomist and the species of the geneticist. Thus Winge (1938), in discussing *Geum rivale* and *G. urbanum,* states that genetically these are one species, since they are perfectly interfertile, but taxonomically they are separate species. Moore (1946) (a zoologist) has written: "The geneticist lays emphasis on the lack of interbreeding with other species under natural conditions. If the morphological differences of the taxonomists and the isolating mechanism of the geneticist arise independently, then taxonomic and genetic species might not coincide. However, if both originate in the same manner, and in some cases probably have the same genetic basis, the usual identity becomes understandable". Mayr (1942) has pointed out that in the earlier definitions of species the difference between crossability and sterility has been overlooked, and more recently he (1948) has reviewed the literature bearing on the nature of species in animals.

In recent years there has been an attempt to bring together the concepts of the geneticist and of the taxonomist in a definition of a species (Clausen, Keck and Hiesey, 1939, 1945). In this classification, which is based upon the pioneer work of Turesson, if the hybrids are sterile or if no hybrids can be produced, the two entities under consideration belong to different species complexes or are "coenospecies" in the terminology of the experimentalists; if the entities hybridize, or can be hybridized, and the hybrids are partially sterile and the second generation weak, they are approximately the "species of moderately conservative taxonomists working along traditional lines" or "ecospecies" of the biosystematists; if the hybrids between the two entities are fertile and the second generation vigorous, the two are merely subspecies or "ecotypes". In this noble attempt at a species definition based on experiment, the authors (1939) point out that there is no absolute gap between ecotypes and ecospecies, although only a few cases are intermediate. They also point out that a small number of the hybrids may be vigorous and fertile and eventually tend to be absorbed into one or the other of the parental species, which would be introgression as it is defined here. For an appraisal of the recent biosystematic trends, the reader is referred to the papers of Turrill (1946)and Valentine (1948).

In an attempt to apply the above definition to the cases reviewed here it can be seen that some of the examples would fall into the category of subspecies (ecotypes), as in *Vaccinium,* whereas others could truly be relegated to the category of species (ecospecies), as in *Helianthus.* A great number of the cases, however, appear to fall somewhere between the two categories. One example calls for further comment. Systematists have for over a hundred years been inclined to regard *Euchlaena* and *Zea* as separate genera. However, as a result of recent cytogenetic studies, *Euchlaena* has been merged with *Zea,* but *Z. mexicana* and *Z. mays* are still retained as distinct species (Reeves and Mangelsdorf, 1942). Clausen, Keck and Hiesey (1945) point out, however, that these two entities can hardly be considered more than ecotypes in view of their complete fertility and lack of weakness in the F_2 generation. *Zea mays* is, of course, a domesticated plant, and it is now apparent that once man brings a plant under cultivation the delimitation of species frequently becomes very difficult.

There is generally a close correlation of morphology with crossibility; Babcock (1947), as the result of years of study on *Crepis,* has found that this holds true for the most part in this genus. Ownbey and Weber (1943) have pointed out, however, that there are just enough exceptions to this conception "to make the strict application of hybrid sterility or inviability to species delimitation as unsatisfactory as the axiomatic application of the traditional criterion, morphological difference".

An interesting situation would arise if strict application of the above scheme of Clausen *et al.* were followed. Many genera would probably become monotypic. In commenting on the reduction of a species of *Hevea* because an intermediate plant had been found connecting it to another species, Baldwin (1947) states: "One might with almost as much reason render the whole genus unispecific".

According to Turrill (1942), the botanist knows that the use of sterility as a criterion for the delimitation of plant species would lead to a special classification of little or no general use. Goodwin (1937*b*) feels that *Solidago rugosa* and *S. sempervirens,* in spite of their hybridization, should be maintained as distinct species "on morphological grounds and for the sake of convenience". Sax and Sax (1947) have stated that it is a matter of taxonomic convenience whether the parents of so-called intergeneric hybrids are placed in the same or different genera. There is quite definitely a practical as well as a biological problem involved in the matter of species definitions. Even with experimental methods, what determines a species still remains largely a matter of judgment.

Whether or not the entities discussed here are considered species, there are strong barriers to prevent their merging into one unit, as will be seen in the next section. Perhaps the ability to remain distinct in spite of hybridization entitles them rightfully to the designation of "species".

BARRIERS TO SWAMPING

It has been generally agreed that there has not been an obliteration of specific boundaries as a result of hybridization in most of the examples which have been critically studied (Ostenfeld, 1928; Marsden-Jones and Turrill, 1928, 1947; Du Rietz, 1930; Allard, 1932; Anderson, 1936*b*; Ownbey and Weber, 1943; Epling, 1947; Stebbins, Matske and Epling, 1947; Heiser, 1947, 1949; Anderson, 1948; Valentine, 1948). What are the barriers to the amalgamation of two species into one as the result of hybridization?

First, it is apparent that those internal and external isolation barriers normally present between species may operate in part (see Dobzhansky, 1941; Stebbins, 1942; and Cain, 1944, for a discussion of isolation barriers). Partial sterility is one such barrier. The initial sterility between species of annual sunflowers is rather great (Heiser, 1947, 1949), and partial sterility seems to exercise an isolating effect in *Salvia* (Epling, 1947). It is probable that hybridization may lead to the development of complete sterility barriers between some species (Dobzhansky, 1941), but as to whether or not this is a general phenomenon is still a matter of conjecture.

Among the other barriers which may operate is a partial difference in time of blooming (Anderson, 1936*c*; Erickson, 1945; Heiser, 1947) and mechanical differences in the structure of the flowers (Epling, 1947*b*). Ownbey and Weber (1943) have suggested that "possibly for reasons of time and distance alone" there is no submerging of incompletely isolated species of *Balsamorhiza*. In *Primula vulgaris* and *P. elatior,* two homoploid species, apparently unbalance in the endosperm may prevent hybrids with a normal embryo from reaching maturity (Valentine, 1947).

It was pointed out above that most of the pairs of species involved in hybridization exhibit strongly different ecological preferences and that intermediate types of habitat are necessary for the success of the hybrid derivatives. Adaptation of the parent species to a particular habitat is an important factor in keeping species distinct despite hybridization, according to Anderson (1948). He points out that "the second (hybrid) generation will be made up of individuals, each of which requires its own peculiar habitat for optimum development", and that such a great variety of habitats is never encountered in nature. It would thus appear almost impossible for the gap between two species with strongly different habitat preferences to be completely bridged, due to the barrier of the habitat alone. Du Rietz (1930) discusses a number of cases of hybridization in which the species are kept distinct by reason of ecological barriers. Recently Hovanitz (1948) has shown that interfertile species of the butterfly genus *Colias* are separated by such a barrier. *C. eurytheme* is adapted primarily to alfalfa, *C. philodice* to red clover, and there is no amalgamation of the two species into one as a result of the hybridization which is known to take place.

The matter of competition is simply another aspect of the habitat requirements of the hybrids and parents. The poor chances of the hybrids competing with the parents (Ostenfeld, 1928) is thus fully explained, including the slight discrimination against hybrid oak seedlings in nature (Stebbins, Matske and Epling, 1947). It has also been pointed out that the number of hybrids thrown into competition with the parents would be small (Epling, 1947*a*, 1947*b*), which would further serve as a barrier. Epling (1947*a*) maintains that the "morphological constancy of sympatric species may be preserved despite compatibility and hybridization . . . as long as the equilibrium of competition is maintained". From Anderson's (1948) remarks it becomes quite clear why the equilibrium of competition is maintained when two species are adapted to different habitats.

As a result of a study of artificial hybrids between two highly compatible species of *Nicotiana,* Anderson (1939*a*) has come to the conclusion that the recombinations which may appear in the F_2 of any such cross represent only a small fraction of the recombinations theoretically possible. The barriers to the appearances of certain recombinations are as follows: *a*) gametic elimination; *b*) zygotic elimination; *c*) pleiotropy; and *d*) linkage. Anderson states that all of the quantitative characters of an organism are closely linked with one another, and moreover that "the hindrances imposed by linkage of multiple factor characteristics are found to be severe".[4] In another place he (1939*b*) has estimated the total magnitude to gene recombination imposed by linkage, and has recently stated that linkage alone can be a barrier to complete swamping of interfertile species (1948). Dansereau (1938, 1941) and Dansereau and Lafond (1941) have observed that only a small number of the theoretical hybrid combinations in *Cistus* and *Acer* are met with in nature. However, in these examples it is quite possible that certain of the expected recombinations have failed to appear because of limitations of the habitat.

Also worthy of consideration in connection with barriers to swamping is the suggestion that species possess distinct modifier complexes which result in the appearance of inferior segregates when fertile species hybrids are interbred (see Stephens, 1947). For example, the cultivated cotton species, *Gossypium barbadense* and *G.* ~~*bu*~~hi*rsutum*, give fertile F_1 hybrids, and yet the integrity of the two species is preserved even when the two are grown in mixtures (Hutchinson, Silow and Stephens, 1947). Whatever the barrier or barriers to swamping in this case, they appear to be very effective to preserve the identity of these two cultigens.

---

[4] Dempster (Genetics **34**: 272–284. 1949) has recently shown that the effects of linkage in restricting genetic recombination in the F_2 is very slight; however, linkage can increase the variation of F_2 character combinations in the direction of the parental types.

From the above it can be seen that there are a number of factors, acting separately or together, which would be expected to prevent swamping of distinct species in nature. The barriers of the habitat or linkage alone might be expected to prevent the amalgamation of fully compatible species. The examples given above for the most part have been taken from plants, but they probably apply equally well to animals. Blair (1941*a*, 1942) found that the isolating mechanisms preventing swamping in toads are partly seasonal, partly ecological and partly sexual, *i.e.*, a difference in mating calls. Ethnological isolating mechanisms, such as the last mentioned, while common in animals are unknown for plants, and this may explain in part the greater occurrence of hybrids in plants. The barrier imposed by different habitats in animals has been discussed by Mayr (1942).

On the basis of a study of "complex mutations" (mutations affecting both a vegetative and floral character), Lamprecht (1944, 1945) states that genes can be divided into two kinds, intra-specific and inter-specific. The latter genes are species specific and constitute the genic basis of the species barrier; as a result of this he makes the bold statement that it is possible to give a "univocal" definition of a species as a unit including all biotypes which differ from all others by at least one common inter-specific gene. There are only two such genes which separate *Phaseolus vulgaris* and *P. coccineus* (although he states that most species probably differ for a number of inter-specific genes); all of the other genes, intra-specific, of these two species may be recombined into fertile offspring through hybridization, whereas the interspecific genes lead to sterility when placed on the "plasmic" background of the other species. This work, although appearing rather highly speculative to the present reviewer, is of great theoretical importance to introgression. It would follow from Lamprecht's conclusions that introgression of intra-specific genes could readily take place between inter-fertile species, but that the presence of inter-specific genes would always provide a barrier to swamping. Detailed discussion and criticism of this work is out of place in the present article, but in passing, attention should be called to the similarity of Lamprecht's ideas of the origin of a species by a single mutation to those of Goldschmidt. Goldschmidt states (1940, p. 252) that "it does not make any difference whether a single macroevolutionary step is caused by a major change within the chromosome pattern . . . *or by a special kind of gene mutation with generalized effect, if such is imaginable*". (Italics mine.) (See also Castle, 1946, in regard to Lamprecht.)

The possibility that swamping has occurred or may occur cannot be overlooked. Winge (1938) states that the possibility seems to exist that *Geum rivale* and *G. urbanum* in thousands of years may approach one another through hybridization and finally be merged. Dobzhansky (1941) has suggested the merging of two species into a single highly variable one as a result of introgression. Cain (1944) has also suggested that introgression might result in swamping. The possible swamping in *Salix* has been discussed by Du Rietz (1930).

Two examples that have been carefully studied deserve further consideration. Mason (1942) has postulated the genetic submergence of *Pinus remorata* by the ecologically more vigorous *P. muricata* through a process of gene infiltration. This process has taken place over a long period of time, and the conclusions are based on a study of both living and fossil plants. With a changing environment it is apparent that a plant must change to meet the new conditions, migrate or become extinct. It may be that *P. remorata* was faced with such a situation. This brings to mind Dansereau's observation on *Acer* in the Laurentian area mentioned under the section on ecology. The possibility does seem to exist then that swamping may take place over broad expanses of time and in the face of changing environmental conditions.

The second example involves categories below species. According to Woodson (1947), *Asclepias tuberosa* ssp. *tuberosa* is being engulfed by its sister subspecies on each side. The three subspecies differentiated from each other in isolation, and swamping is apparently occurring now that they have come back together. However, the swamping is by no means complete, as is testified by the fact that the three subspecies are fairly well defined throughout most of their ranges. The reasons for the possible swamping should be considered, nevertheless. Subspecies are normally characterized by complete fertility, and there would be no sterility barrier, although, as we have seen, this is also true for some of the "species" discussed above. Secondly, geographic subspecies may not show the strong ecological differences of sympatric species, and thus there might be little or no barrier by means of the habitat. Thirdly, subspecific categories presumably differ from each other by fewer genes and modifier complexes than do species. Hence it is possible that linkage alone would be less of a barrier in crosses between subspecies.

EVOLUTIONARY ROLE

Opinions as to the importance of hybridization in evolution range from those of Lotsy[5] (1916, 1925), who claimed that all evolution is due to hybridization and that mutations do not occur, to those who would assign it a relatively minor role. Lotsy's views on evolution have received few or no adherents, although Turrill (1942) has suggested that there may be more to his theory than most people realize. The attitude that appears to be prevalent today is that hybridization serves to recombine mutations: the "fuel" for evolution being mutations, the "engine" being the genetic system, and the "guiding force" being natural selection (Stebbins, unpubl.).

The possible significance of introgression to evolution has been discussed by a number of writers (Anderson and Hubricht, 1938*a*; Dansereau, 1941; Dobzhansky, 1941; Heiser, 1949; and Anderson, 1949). One of the first effects of introgression is an enrichment of variability of the species concerned. Mangelsdorf (1947) states that new variability "with almost explosive force" must have come into being in a relatively short time as a result of the hybridization of *Zea* and *Tripsacum*. Clausen, Keck and Hiesey (1947) have written: "when crossing occurs between contrasting geographic or ecologic races, so many new forms appear that the variability may often surpass that in wild populations of the species . . .". Secondly, and directly related to the first, hybridization offers advantages for plasticity, though at a sacrifice of immediate fitness. The opportunities afforded by hybridization for flexibility keep the species in a dynamic rather than a static condition, and it may be that the most rapid evolution occurs in such species. From out of such hybridization superior genotypes might emerge, better fit to new environments (Dobzhansky, 1941; Epling, 1947*a*, 1947*b*; Heiser, 1947, 1949). For every superior genotype produced, however, there are probably numerous misfits or sterile plants, so that hybridization between species may also be a destructive process. "The final result (of hybridization) will depend upon the balance between the deleterious effects of the foreign germ plasm and its advantageous effects in areas where the hybridization has taken place or to which the hybrids may spread" (Anderson and Hubricht, 1938*a*).

---

[5] The idea of evolution by means of hybridization did not originate with Lotsy (see Du Rietz, 1930, for a brief historical account of the idea).

The possibility of the creation of new taxonomic entities by hybridization (without polyploidy) also deserves consideration. There is strong circumstantial evidence that subspecific entities have been created by such a process, for example, in *Juniperus* (Fassett, 1944, 1945), in *Cistus* (Dansereau, 1941) and in *Hevea* (Baldwin, 1947; Seibert, 1947), and it is possible of course that some of these may represent incipient species. Wiegand in 1935 wrote that it was not yet clear whether a new species could be created by hybridization. Most botanists will now concede that such may arise by hybridization followed by polyploidy, although it is still not clear whether a species may arise from hybridization at a homoploid level. Lamprecht (1941), through the crossing of two species of *Phaseolus,* obtained fertile plants in later generations which were partially sterile when backcrossed to the parent species, which might be called species. Lewis and Epling (1946) and Epling (1947*a*) have recently postulated the origin of a species of *Delphinium* through hybridization at a diploid level.

Interestingly enough the theory of the origin of cultivated plants by hybridization goes all the way back to Linnaeus, and selection from the products of inter-specific hybridization, unaccompanied by chromosome duplication or alteration, has recently been given as one of the four modes of origin of cultivated plants (Crane, 1939). One of the most striking examples of the influence of hybridization on a cultivated plant is seen in maize. Hybridization between *Zea* and *Tripsacum* is thought to have greatly modified the former plant throughout the northern area of its range (Mangelsdorf and Reeves, 1939; Mangelsdorf, 1947). The possible importance of introgression in the evolution of cultivated plants has been stressed by Griggs (1937) and by Anderson (1949); it is highly desirable that more studies on the origin of cultivated plants be undertaken with due consideration to a possible role of introgression.

One final important point requires discussion here. If a "new" species is created through hybridization, has anything really *new* been created in the process? Some years ago Wiegand (1935) raised this same question and seemed to feel that only recombinations and blends are created as a result of hybridization. Anderson (1939*a*), as a result of experimental studies with two species of *Nicotiana,* has stated that hybridization simply recombines previous genetic material and that no new characters are created in the process. It is also generally assumed that hybridization leading to polyploidy is not a progressive evolutionary factor from the standpoint of the origin of new genetic material (Stebbins, 1940). Lotsy (1916, 1925), on the other hand, believed that all new characters arise as a result of crossing. Part of the controversy centers on how one would define a new character, and exactly what constitutes a character is, as Allan (1931) has put it, very elusive. It is now known that the interaction of genes may produce what may be termed a "new" character, although no new genetic material has actually been formed. It is also now known that hybridization, at least in some instances, may stimulate the mutation rate. Classic examples are found in *Drosophila* and *Gossypium.* The literature and other examples may be found in Harland (1937) and Dobzhansky (1941). Thus truly new characters backed by new genic material may arise indirectly as a result of crossing.

In conclusion we may state that it is still too early to evaluate fully the precise role of hybridization in evolution. That it does play a role there can be no doubt, although that role may be a secondary one. It has been stated that "mutation and hybridization may have worked hand in hand to produce regional diversity" (Clausen, Keck and Hiesey, 1940), and that it will require exceedingly critical data to demonstrate the relative importance of the two processes (Anderson and Hubricht, 1938*a*).

TABLE 1

SOME PROBABLE CASES OF INTROGRESSION IN HIGHER PLANTS[1]

Family and Species	2n Chromosome Number[2]	Chief Lines of Inquiry Other Than Morphological						Reference
		1	2	3	4	5	6	
		Statistical	Ecological or Geographical	Meiotic Behavior	Pollen or Seed Counts	Artificial Crosses	Progeny Tests	
CUPRESSACEAE								
Juniperus horizontalis	..	x	x					
scopulorum	..							
scopulorum	..	x	x					Fassett, 1944, 1945
virginiana	(22)							
horizontalis	..	x	x	(+)				(+ Ross & Duncan,
virginiana	(22)							unpubl.)
POTOMOGETONACEAE								
Potomogeton spp.								Ogden, 1943
GRAMINEAE								
**Zea mays*	(20)							
Tripsacum sp.	..							
Zea mays	(20)	x	x	x	x	x		Mangelsdorf & Reeves,
mexicana	(20)							1939; Mangelsdorf, 1947

TABLE 1—(*Continued*)

Family and Species	2n Chromosome Number[2]	Chief Lines of Inquiry Other Than Morphological						Reference
		1	2	3	4	5	6	
		Statistical	Ecological or Geographical	Meiotic Behavior	Pollen or Seed Counts	Artificial Crosses	Progeny Tests	
Poa glauca var. *glaucantha*	(42, 45, 65, 70–72)				x			Butters & Abbe, 1947
nemoralis var. *interior*	(28, 42)							
Elymus canadensis	(28)							Booker & Tryon, 1948
Hystrix patula	(28)							
**Bromus inermis*	56	x	x	x	x	x		
pumpellianus	56							
inermis	56	x	x	x	x	x		
riparius	70							
pumpellianus	56	x	x	x	x	x		Elliott, unpubl.
riparius	70							
LILIACEAE								
Calochortus nudus	(20)		x					Ownbey, 1940
minimus	. .							
COMMELINACEAE								
**Tradescantia virginiana*	24	x	x	(x)[3]	(x)		(x)	Anderson, 1936*c*
canaliculata	24							

TABLE 1—(*Continued*)

Family and Species	2n Chromosome Number[2]	Chief Lines of Inquiry Other Than Morphological						Reference
		1	2	3	4	5	6	
		Statistical	Ecological or Geographical	Meiotic Behavior	Pollen or Seed Counts	Artificial Crosses	Progeny Tests	
canaliculata	24	x	x	x	x	x	(x)	
subaspera	24							
humilis	12	x	x	x	x	x	(x)	Anderson & Sax, 1936
canaliculata	12							
canaliculata	12, 24	x	x	(x)	(x)	(x)		
occidentalis	12, 24							
canaliculata	24	x	x	(x)	(x)			Anderson & Hubricht, 1938*a* (see also Anderson & Woodson, 1935; Anderson, 1936*d*, 1949 Riley, 1939*c*
bracteata	24							
canaliculata	24	x	x	x	x			
hirsutiflora	24							
paludosa	12							
IRIDACEAE								
**Iris fulva*	42	x	x		x			Viosaca, 1935
hexagona var. *giganticaerulea*	42, 44							Foster, 1937 Riley, 1938, 1939*a*, *b*.

TABLE 1—(*Continued*)

Family and Species	Chromosome 2n Number[3]	Chief Lines of Inquiry Other Than Morphological						Reference
		1	2	3	4	5	6	
		Statistical	Ecological or Geographical	Meiotic Behavior	Pollen or Seed Counts	Artificial Crosses	Progeny Tests	
ORCHIDACEAE								
Orchis incarnata	(40, 80)		x					Pettersson, 1947
maculata	(40, 80)							
FAGACEAE								
**Quercus ilicifolia*	(48)	x	x					Stebbins, Matske & Epling, 1947
marilandica	(48)							
RANUNCULACEAE								
**Clematis Pitcheri*								
Fremontii var.	16		x	x				Erickson, 1945
Riehlii	16							
CARYOPHYLLACEAE								
Melandrium album	24		x		x	x		Baker, 1947 & unpubl.
dioicum	24							
Silene cucubalus								
(*vulgaris*)	24	x	x			x	x	Marsden-Jones & Turrill, 1928–1947 (see also Turrill, 1946)
maritima	24							

TABLE 1—(*Continued*)

Family and Species	2n Chromosome Number[a]	Chief Lines of Inquiry Other Than Morphological						Reference
		1	2	3	4	5	6	
		Statistical	Ecological or Geographical	Meiotic Behavior	Pollen or Seed Counts	Artificial Crosses	Progeny Tests	
LEGUMINOSAE								
Baptisia leucantha	..	x	x		x			Larisey, 1940
viridis	..							
EUPHORBIACEAE								
**Hevea brasiliensis*	36	x	x					
pauciflora	36							
brasiliensis	36		x					Baldwin, 1947; Seibert, 1947, 1948
spruceana	36							
and other species								
ACERACEAE								
**Acer saccharophorum*	(26)	x	x					Dansereau & Lafond, 1941; —— & Desmarais, 1947
nigrum	(26)							
RHAMNACEAE								
Ceanothus papillosus	24	x	x					
thyrsiflorus	24							

TABLE 1—(*Continued*)

Family and Species	2n Chromosome Number[a]	Chief Lines of Inquiry Other Than Morphological 1 Statistical	2 Ecological or Geographical	3 Meiotic Behavior	4 Pollen or Seed Counts	5 Artificial Crosses	6 Progeny Tests	Reference
prostratus	24	x	x					
cuneatus	24							
and other species								McMinn, 1944
CISTACEAE								
**Cistus laurifolius*	(18)	x	x					
ladaniferus	18							
monspeliensis	18	x	x					Dansereau, 1941 (see also Dansereau, 1938, 1938a, b, c, 1943)
salvifolius	18							
and other species								
ERICACEAE								
Vaccinium torreyanum	24							
atrococcum	24		x					
angustifolium	24							
and other species						x		Camp, 1942; Darrow & Camp, 1945
PRIMULACEAE								
Primula elatior	(22)	x	x		x	x		Valentine, 1947, 1948
vulgaris	(22)							

TABLE 1—(*Continued*)

Family and Species	2n Chromosome Number[a]	Chief Lines of Inquiry Other Than Morphological						Reference
		1	2	3	4	5	6	
		Statistical	Ecological or Geographical	Meiotic Behavior	Pollen or Seed Counts	Artificial Crosses	Progeny Tests	
OLEACEAE								
Fraxinus pallisae	..	x						Anderson & Turrill, 1938
oxycarpa	..							
APOCYNACEAE								
Apocynum cannabinum	(16?)	x			x		x	Anderson, 1936*a*
androsaemifolium	(16?, 22)							
LABIATAE								
Salvia apiana	32	x	x		x			Epling, 1947*b*
mellifera	32							
RUBIACEAE								
**Cinchona* spp.			x					Camp, 1948
COMPOSITAE								
Aster multiflorus	10	x	x	x	x	x	x	Wetmore & Delisle, 1939
novae-angliae	10							
Balsamorhiza careyana	..		x					Ownbey & Weber, 1943
deltoidea	..							
and possibly other species								
Centaurea nigra	44							Marsden-Jones & Turrill 1930*a*, *b*, 1937 (a detailed report has not yet been publ.)
jacea	44							

Family and Species	Number[3] 2n Chromosome	Chief Lines of Inquiry Other Than Morphological						Reference
		1	2	3	4	5	6	
		Statistical	Ecological or Geographical	Meiotic Behavior	Pollen or Seed Counts	Artificial Crosses	Progeny Tests	
**Helianthus annuus*	34	x	x	x	x	x	x	Heiser, 1947
petiolaris	34							
annuus	34	x	x	x	x	x	x	Heiser, 1949
bolanderi	34							
**Parthenium argentatum*	36, 54, 72, 108			x		x	x	Rollins, 1944, 1945*a, b*
incanum	54, 63, 72, 90, 108							
Solidago rugosa	18	x	x	x	x	x	x	Goodwin, 1937*a, b*
sempervirens	18							
Vernonia crinita								Steyermark, 1939
baldwini								
altissima								
missurica								
fasciculata								
Wyethia amplexicaulis	38		x					Weber, 1946
helianthoides	38							
and other species								

---

[1] Those examples in which the hybridization has been defined as introgression are preceded by an asterisk (*).

[2] The chromosome numbers have been taken from the works cited except where the number is given in parentheses. These latter counts have been taken from Darlington and Janaki-Ammal (1946) or Myers (1947), except for *Acer nigrum*, the count for which has been made by the author (unpubl.).

[3] Parentheses are used where the data are unpublished.

LITERATURE CITED

ALLAN, H. H. The significance of hybridism in the New Zealand Flora. Rep. 20th Meet. Aust. & N. Z. Assn. Adv. Sci. **20**: 429–477. 1931.

————. Wild species-hybrids in the phanerogams. Bot. Rev. **3**: 593–615. 1937.

————. The nomenclature of hybrids. Chron. Bot. **5**: 205–209. 1939.

————. Natural hybridization in relation to taxonomy. *In* J. Huxley, The New Systematics. Pp. 515–528. 1940.

————. Wild species-hybrids in the phanerogams. II. Bot. Rev. **15**: 77–105. 1949.

ALLARD, H. A. A progeny study of the so-called oak species *Quercus saulii*, with notes on other probable hybrids found in or near the District of Columbia. Bull. Torrey Bot. Club **59**: 267–277. 1932.

ANDERSON, E. An experimental study of hybridization in the genus *Apocynum*. Ann. Mo. Bot. Gard. **23**: 159–168. 1936*a*.

————. The species problem in *Iris*. Ann. Mo. Bot. Gard. **23**: 457–509. 1936*b*.

————. Hybridization in American Tradescantias. Ann. Mo. Bot. Gard. **23**: 511-525. 1936*c*.

————. A morphological comparison of triploid and tetraploid interspecific hybrids in *Tradescantia*. Genetics **21**: 61–65. 1836*d*.

————. Recombination in species crosses. Genetics **24**: 668–698. 1939*a*.

————. The hindrance to gene recombination imposed by linkage: An estimate of its total magnitude. Am. Nat. **73**: 185–188. 1939*b*.

————. The technique and use of mass collections in plant taxonomy. Ann. Mo. Bot. Gard. **28**: 287-292. 1941.

————. The hybridization of the habitat. Evolution **2**: 1–9. 1948.

————. Introgressive hybridization. Biol. Mem. 1949.

———— and D. G. DIEHL. Contributions to the *Tradescantia* problem. Jour. Arn. Arb. **8**: 213–231. 1932.

———— and L. HUBRICHT. Hybridization in *Tradescantia*. III. The evidence for introgressive hybridization. Am. Jour. Bot. **25**: 396–402. 1938*a*.

———— and ————. The American sugar maples. I. Phylogenetic relationship as deduced from a study of leaf variation. Bot. Gaz. **100**: 312–323. 1838*b*.

———— and K. SAX. A cytological monograph of the American species of *Tradescantia*. Bot. Gaz. **97**: 433–476. 1936.

———— and W. B. TURRILL. Biometrical studies on herbarium material. Nature **136**: 986. 1935.

———— and ————. Statistical studies on two populations of *Fraxinus*. New Phyt. **37**: 160–172. 1938.

———— and R. E. WOODSON, JR. The species of *Tradescantia* indigenous to the United States. Contr. Arn. Arb. **9**: 1–132. 1935.

BABCOCK, E. B. Cyto-genetics and the species-concept. Am. Nat. **65**: 5–18. 1931.

————. Cytogenetics and speciation in *Crepis*. *In* M. Demerec, Advances in Genetics, Vol. 1: 69–93. 1947.

BAKER, H. G. *Melandrium*. Jour. Ecol. **35**: 271–283. 1947.

————. Criteria of hybridity. Nature **159**: 221–223. 546. 1947.

BALDWIN, J. T., JR. *Hevea:* a first interpretation. Jour. Hered. **38**: 54–64. 1947.

BENEDICT, R. C. Problems and objectives in the study of fern hybrids. Am. Fern Jour. **35**: 65–76. 1945.

BLACK, J. D. and L. O. WILLIAMSON. Artificial hybrids between muskellunge and northern pike. Trans. Wis. Acad. Sci. **38**: 299–314. 1946.

Black, R. A. Description of a new eucalypt hybrid. X. *Eucalyptus radiodives* ("Buttercup Peppermint") (*radiata* × *dives*) R. A. Black, hybrid nov. Victorian Nat. **60:** 175. 1944.

Blair, A. P. Variation, isolating mechanisms, and hybridization in certain toads. Genetics **26:** 398–417. 1941*a*.

———. Isolating mechanisms in tree frogs. Proc. Nat. Acad. Sci. **27:** 14–17. 1941*b*.

———. Isolating mechanisms in a complex of four species of toads. Biol. Symp. **6:** 235–249. 1942.

Booker, L. E. and R. M. Tryon. A study of *Elymus* in Minnesota. Rhodora **50:** 80–91. 1948.

Brainerd, E. Hybridism in the genus *Viola*. II. Rhodora **8:** 6–10. 1906.

Brett, R. G. A survey of Eucalypt species in Tasmania. Pap. & Proc. Royal Soc. Tasmania **1937:** 76–109. 1938.

Butters, F. K. and E. C. Abbe. The genus *Poa* in Cook County, Minnesota. Rhodora **49:** 1–21. 1947.

Cain, S. A. Foundations of plant geography. 1944.

Camp, W. H. Studies in the Ericales. A discussion of the genus *Befaria* in North America. Bull. Torrey Bot. Club **68:** 100–111. 1941.

———. On the structure of populations in the genus *Vaccinium*. Brittonia **4:** 189–204. 1942.

———. Cinchona at high altitudes in Ecuador. Brittonia **6:** 394–430. 1948.

——— and C. L. Gilly. The structure and origin of species. Brittonia **4:** 323–385. 1943.

Castle, W. E. Genes which divide species or produce hybrid vigor. Proc. Nat. Acad. Sci. **32:** 145–149. 1946.

Chapin, J. P. Variation and hybridization among the paradise flycatchers of Africa. Evolution **2:** 111–126. 1948.

Clausen, J. Studies on the collective species *Viola tricolor* L. II. Bot. Tidsk. **37:** 363–416. 1922.

———, *et al.* The concept of species based on experiment. Am. Jour. Bot. **26:** 103–106. 1939.

———, *et al.* Experimental studies on the nature of species. I. Effect of varied environments on western North American plants. Carnegie Inst. Wash., Pub. 520: 1–452. 1940.

———, *et al.* Experimental studies on the nature of species. II. Plant evolution through amphiploidy and autoploidy, with examples from the Madiinae. Carnegie Inst. Wash., Pub. 564: 1–174. 1945.

———, *et al.* Heredity of geographically and ecologically isolated races. Am. Nat. **81:** 114–133. 1947.

Cleland, R. E. The problem of species in *Oenothera*. Am. Nat. **78:** 5–28. 1944.

Cockayne, L. Hybridism in the New Zealand flora. New Phyt. **22:** 105–127. 1923.

———. Hybridism in the forests of New Zealand. Acta Forest. Fenn. **34:** 23 pp. 1929.

——— and H. H. Allan. An annotated list of groups of wild hybrids in the New Zealand flora. Ann. Bot. **48:** 1–55. 1934.

Cole, L. J. and R. M. Shackelford. Fox hybrids. Trans. Wis. Acad. Sci. **38:** 315–332. 1946.

Crane, M. B. The origin and behavior of cultivated plants. *In* J. Huxley, The New Systematics. Pp. 529–547. 1939.

Cugnac, A. de Sur les conséquences systématiques de la dominance et de recéssivité des caractères spécifiques dans les croisements. Compt. Rend. Acad. Sci. Paris **211:** 268–270. 1940.

Dansereau, P. Études sur les hybrides de Cistes. III. Statistiques des caractères du *Cistus monspeliensis* L. Bull. Soc. Bot. France **85:** 668–678. 1938.

———. Monographie du genre *Cistus* L. Boissiera **4:** 1–90. 1939*a*.

————. Sens de l'hybridation dans le genre *Cistus*. Ber. Schweiz. Bot. Ges. **49**: 412–413. 1939*b*.

————. Definizione del genere *Halimiocistus*. Nuovo Gior. Bot. Ital. **46**: 357–360. 673. 1939*c*.

————. Études sur les hybrides de Cistes. Ann. Épiphyt. et Phytogén. **6**: 7–26. 1940.

————. Études sur les hybrides de Cistes. IV. Correlation des caractères du *C. salviifolius* L. VI. Introgression dans la section Ladanium. Canad. Jour. Res., C., **19**: 27–39. 59–67. (Contr. Inst. Bot. Univ. Montreal, 38.) 1941.

————. Études sur les hybrides de Cistes. V. Le comportement du *x C. florentinus* Lam. Candollea **10**: 9–22. 1943.

———— and Y. Desmarais. Introgression in sugar maples. II. Am. Midl. Nat. **37**: 146–161. 1947.

———— and A. Lafond. Introgression des caractères de *l'Acer saccharophorum* K. Koch et de *l'Acer nigrum* Michx. Contr. Inst. Bot. Univ. Montreal **37**: 15–31. 1941.

Darrow, G. M. and W. H. Camp. *Vaccinium* hybrids and the development of new horticultural material. Bull. Torrey Bot. Club **72**: 1–21. 1945.

Darlington, C. D. What is a hybrid? Jour. Hered. **28**: 308. 1937a.

————. Recent advances in cytology. 2nd ed. 1937*b*.

———— and E. K. Janaki Ammal. Chromosome atlas of cultivated plants. 1945.

Darwin, C. The origin of species. 6th ed. 1872.

Davidson, J. F. The polygonal graph for simultaneous portrayal of several variables in population analysis. Madroño **9**: 105–110. 1947.

Dobzhansky, Th. Genetics and the origin of species. Rev. ed. 1941.

Du Rietz, G. E. The fundamental units of biological taxonomy. Svensk. Bot. Tidskr. **24**: 333–428. 1930.

Elliott, F. C. Cross-fertility and cytogenetics of selected *Bromopsis* section members within the genus *Bromus* L. [Unpubl.]

Epling, C. Actual and potential gene flow in natural populations. Am. Nat. **81**: 104–113. 1947*a*.

————. Natural hybridization of *Salvia apiana* and *S. mellifera*. Evolution **1**: 69–78. 1947*b*.

Erickson, R O. The *Clematis Fremontii* var. *Riehlii* population in the Ozarks. Ann. Mo. Bot. Gard. **32**: 413–460. 1945.

Fagerlind, F. Embryologische, cytologishe und bestäubungsexperimentelle Studien in der Familie Rubiaceae nebst Bemerkungen über einige Polyploidistätsprobleme. Acta Horti Bergiana **11**: 195–470. 1937.

Fassett, N. C. *Juniperus virginiana, J. horizontalis,* and *J. scopulorum*. I. The specific characters. Bull. Torrey Bot. Club **71**: 410–418. 1944. II. Hybrid swarms of *J. virginiana* and *J. scopulorum. ibid.* 475–483. III. Possible hybridization of *J. horizontalis* and *J. scopulorum. ibid.* **72**: 42–46. 1945. IV. Hybrid swarms of *J. virginiana* and *J. horizontalis. ibid.* 379–384. V. Taxonomic treatment. *ibid.* 480–482.

Focke, W. O. Die Pflanzenmischlinge, ein Beitrag zur Biologie der Gewächse. 1881.

Fosberg, F. R. Taxonomy and hybridism. Chron. Bot. **5**: 397–398. 1939.

————. For an open-minded taxonomy. Chron. Bot. **6**: 368–370. 1941.

Foster, R. C. A cyto-taxonomic survey of the North American species of *Iris*. Contr. Gray Herb. No. 119: 3–82. 1937.

Friesner, R. C. Two *Solidago* hybrids worthy of names. Butler Univ., Bot. Stud. **6**: 81–83. 1943.

Goldschmidt, R. The material basis of evolution. 1940.

Goodspeed, T. H. and M. V. Bradley. Amphidiploidy. Bot. Rev. **8**: 271–316. 1942.

GOODWIN, R. H. Notes on the distribution and hybrid origin of *x Solidago asperula*. Rhodora **39**: 22–28. 1937*a*.

————. The cytogenetics of two species of *Solidago* and its bearing on their polymorphy in nature. Am. Jour. Bot. **24**: 425–432. 1937*b*.

GORDON, M. Interchanging genetic mechanisms for sex determination. Jour. Hered. **37**: 307–320. 1946*a*.

————. Introgressive hybridization in domesticated fishes. I. The behavior of comet, a *Platypoecilus maculatus* gene in *Xiphophorus helleri*. Zoologica **30**: 77–88. 1946*b*.

————. Speciation in fishes. Distribution in time and space of seven dominant multiple alleles in *Platypoecilus maculatus*. *In* M. Demerec, Advances in Genetics Vol. 1: 95–132. 1947.

GRIGGS, R. F. Hybridity as a factor in evolution. Jour. Wash. Acad. Sci. **27**: 329–343. 1937.

HARLAND, S. C. The genetical conception of the species. Biol. Rev. **11**: 83–112. 1936.

————. The genetics of cotton. XVII. Increased mutability of a gene in *G. purpurascens* as a consequence of hybridization with *G. hirsutum*. Jour. Genet. **34**: 153–168. 1937.

HEISER, C. B., JR. Hybridization between the sunflower species *Helianthus annuus* and *H. petiolaris*. Evolution **1**: 249–262. 1947.

————. An evolutionary study of the sunflower species *Helianthus annuus* and *H. Bolanderi*. Univ. Calif. Publ. Bot. **23**: 157–208. 1949.

———— and T. W. WHITAKER. Chromosome number, polyploidy, and growth habit in California weeds. Am. Jour. Bot. **35**: 179–186. 1948.

HIORTH, G. Genetische Versuche mit *Collinsia*. IV. Die Analyse eines nahezu sterilen Artbastardes. I. Teil. Die diploiden Bastarde zwischen *Collinsia bicolor* und *C. bartisaefolia*. Zeits. Ind. Abst. Vererb. **66**: 106–157. 1933.

HOVANITZ, W. Ecological segregation of interfertile species of *Colias*. Ecology **29**: 461–469. 1948.

HUBBS, C. L. Speciation of fishes. Am. Nat. **74**: 198–211. 1940.

———— and L. C. HUBBS. Natural hybrids between two species of Catostomid fishes. Pap. Mich. Acad. **31**: 147–167. 1945.

————, *et al.* Hybridization in nature between species of Cantostomid fishes. Contr. Lab. Vert. Biol. Univ. Mich. No. 22: 1–76. 1943.

———— and K. KURONUMA. Hybridization in nature between two genera of flounders in Japan. Pap. Mich. Acad. Sci. **27**: 267–306. 1941.

———— and R. R. MILLER. Mass hybridization between two genera of Cyprinid fishes in the Mohave desert, California. Pap. Mich. Acad. Sci. **28**: 343–378. 1942.

————, *et al.* Hybridization in nature between species of American Cyprinodont fishes. Contr. Lab. Vert. Biol. Univ. Mich. No. 23: 1–21. 1943.

HUBRICHT, L. and E. ANDERSON. Vicinism in *Tradescantia*. Am. Jour. Bot. **28**: 957. 1941.

HUSKINS, C. L. Criteria of hybridity. Science **69**: 399–400. 1929.

HUTCHINSON, J. B. *et al.* The Evolution of *Gossypium*, and the differentiation of the cultivated cottons. 1947.

KOELREUTER, J. G. Vorläufige Nachricht von einigen das Geschlecht der Pflanzen. (1761–1766). Ost. Klas. Exak. Wiss. 41. 1893.

LARISEY, M. M. Analysis of a hybrid complex between *Baptisia leucantha* and *Baptisia viridis* in Texas. Am. Jour. Bot. **27**: 624–628. 1940.

LAMPRECHT, H. Die Artgrenze zwischen *Phaseolus vulgaris* L. und *multiflorus* Lam. Hereditas **27**: 51–175. 1941.

————. Die genisch-plasmatische Grundlage der Artbarriere. Agr. Hort. Genet. **2**: 75–142. 1944.

———. Intra- and inter-specific genes. Agr. Hort. Genet. **3:** 45–60. 1945.

LEWIS, H. and C. EPLING. Formation of a diploid species of *Delphinium* by hybridization. Am. Jour. Bot. **23:** 21s–22s. 1946. [Abs.]

LOTSY, J. P. Evolution by means of hybridization. 1916.

———. Evolution considered in the light of hybridization. 1925.

MCMINN, H. E. The importance of field hybrids in determining species in the genus *Ceanothus*. Proc. Calif. Acad. Sci. **25:** 323–356. 1944.

MANGELSDORF, P. C. The origin and evolution of maize. *In* M. Demerec, Advances in genetics Vol. 1: 161–207. 1947.

——— and R. G. REEVES. The origin of Indian corn and its relatives. Texas Agr. Exp. Sta., Bull. 574: 1–315. 1939.

MARSDEN-JONES, E. M. The genetics of *Geum intermedium* Willd. haud Ehrh. and its back-crosses. Jour. Genet. **23:** 377–395. 1930.

——— *et al.* Special herbaria as adjuncts to modern botanical research. Jour. Ecol. **18:** 379–383. 1930.

——— and W. B. TURRILL. Researches on *Silene maritima* and *S. vulgaris*. I–XXVI. Kew Bull. 1928–1947.

——— and ———. Species studies in plants. Rep. Bot. Exchange Cl. **1930:** 416–420. 1930*a*.

——— and ———. Hybridization in certain genera of the British Flora. [Abs.] Proc. Linn. Soc. London. 141st sess., p. 36. 1930*b*.

——— and ———. Genetical studies in *Centaurea scabiosa* L. and *Centaurea collina* L. Jour. Genet. **34:** 487–495. 1937.

MASON, H. L. Evidence from the fossil record and from the modern distribution for the genetic submergence of *Pinus remorata* by *Pinus muricata*. [Abs.] Committee on Geology and Geography, Rep. of the Subcommittee on Common Problems of Genetics and Paleontology (mimeographed). Nat. Res. Council. 1942.

MAYR, E. Systematics and the Origin of species. 1942.

———. The bearing of the new systematics on genetical problems. The nature of species. *In* M. Demerec, Advances in genetics. Vol. 2: 205–237. 1948.

MOORE, J. A. Incipient intraspecific isolating mechanisms in *Rana pipiens*. Genetics **31:** 304–326. 1946.

MOSS, C. E. Some natural hybrids of *Clematis, Anemone,* and *Gerbera* from the Transvaal. [Abs.] Proc. Linn. Soc. Lond., 141st sess., pp. 36–38. 1930.

MYERS, W. M. Cytology and genetics of forage grasses. Bot. Rev. **13:** 319–421. 1947.

OGDEN, E. C. The broad-leaved species of *Potamogeton* of North America north of Mexico. Rhodora **45:** 57–105, 119–163, 171–214. 1943.

OSTENFELD, C. H. The present state of knowledge on hybrids between species of flowering plants. Masters Lectures, 1927. Jour. Royal Hort. Soc. **53:** 31–44. 1928.

OWNBEY, M. A monograph of the genus *Calochortus*. Ann. Mo. Bot. Gard. **27:** 371–560. 1940.

——— and W. A. WEBER. Natural hybridization in the genus *Balsamorhiza*. Am. Jour. Bot. **30:** 179–187. 1943.

PETTERSSON, B. On some hybrid populations of *Orchis incarnata* x *maculata* in Gotland. Svensk Bot. Tidskr. **41:** 115–140. 1947.

POPOV, M. G. The geographic-morphological method of systematics and the hybridization process in nature. Bull. Appl. Bot., Genet. & Pl. Breed. **17**^{1}: 221–290. 1927. [Eng. summary.]

REEVES, R. G. and P. C. MANGELSDORF. A proposed taxonomic change in the tribe Maydeae (family Gramineae). Am. Jour. Bot. **29:** 815–817. 1942.

RICKETT, H. W. and W. H. CAMP. The nomenclature of hybrids. Bull. Torrey Bot. Club **75:** 496–501. 1948.

RILEY, H. P. A character analysis of colonies of *Iris fulva, I. hexagona* var. *giganticaerulea* and natural hybrids. Am. Jour. Bot. **25:** 727–738. 1938.

————. The problem of species in Louisiana Irises. Bull. Am. Iris Soc. July. 5 pp. 1939*a*.

————. Pollen fertility in *Iris*. Jour. Hered. **30:** 481–483. 1939*b*.

————. Introgressive hybridization in a natural population of *Tradescantia*. Genetics **24:** 753–769. 1939*c*.

————. Introduction to genetics and cytogenetics. 1948.

ROBERTS, H. F. Plant hybridization before Mendel. 1929.

ROLLINS, R. C. Evidence for natural hybridity between guayule (*Parthenium argentatum*) and mariola (*Parthenium incanum*). Am. Jour. Bot. **31:** 93–99. 1944.

————. Some known and probable levels of reciprocal introgression between guayule (*Parthenium argentatum*) and mariola (*P. incanum*). [Abs.] Genetics **30:** 18–19. 1945*a*.

————. Interspecific hybridization in *Parthenium*. I. Crosses between guayule (*P. argentatum*) and mariola (*P. incanum*). Am. Jour. Bot. **32:** 395–404. 1945*b*.

ROSS, J. G. and R. E. DUNCAN. Cytological evidences of hybridization between *Juniperus virginiana* and *J. horizontalis*. [Unpubl.]

SAUER, C. O. Early relations of man to plants. Geog. Rev. **37:** 1–25. 1947.

SAX, H. J. and K. SAX. The cytogenetics of generic hybrids of *Sorbus*. Jour. Arn. Arb. **28:** 137–140. 1947.

SEIBERT, R. J. A study of *Hevea* (with its economic aspects) in the Republic of Peru. Ann. Mo. Bot. Gard. **34:** 261–352. 1947.

————. The uses of *Hevea* for food in relation to its domestication. Ann. Mo. Bot. Gard. **35:** 117–121. 1948.

SHOWALTER, H. M. The distribution of *Tradescantia* in the eastern Tennessee region. Jour. Tenn. Acad. Sci. **13:** 253–258. 1938.

SMALL, J. K. and E. J. ALEXANDER. Botanical interpretation of the iridaceous plants of the Gulf States. Contr. New York Bot. Gard. No. 327: 325–357. 1931.

SPRAGUE, T. A. The nomenclature of hybrids. Chron. Bot. **5:** 209–212. 1939.

STEBBINS, G. L., JR. The significance of polyploidy in plant evolution. Am. Nat. **75:** 54–66. 1940.

————. The role of isolating mechanisms in the differentiation of plant species. Biol. Symp. **6:** 217–233. 1942.

————. The cytological analysis of species hybrids. II. Bot. Rev. **11:** 463–486. 1945.

————. Types of polyploids: their classification and significance. *In* M. Demerec, Advances in genetics **1:** 403–429. 1947*a*.

————. Evidence of rates of evolution from the distribution of existing and fossil plant species. Ecol. Mon. **17:** 149–158. 1947*b*.

————, *et al.* Hybridization in a population of *Quercus marilandica* and *Quercus ilicifolia*. Evolution **1:** 79–88. 1947.

STEPHENS, S. G. Cytogenetics of *Gossypium* and the problem of the origin of new world cottons. *In* M. Demerec, Advances in genetics, Vol. 1: 431–442. 1947.

STEYERMARK, C. S. Distribution and hybridization of *Vernonia* in Missouri. Bot. Gaz. **100:** 548–562. 1939.

SWEADNER, W. R. Hybridization and the phylogeny of the genus *Platysamia*. Ann. Carnegie Mus. **25:** 163–242. 1937.

TURRILL, W. B. The plant-life of the Balkan Peninsula. 1929.

————. The correlation of morphological variation with distribution in some species of *Ajuga*. New Phyt. **33:** 218–230. 1934.

———. The expansion of taxonomy with special reference to the Spermatophyta. Biol. Rev. **13:** 342–373. 1938.

———. Taxonomy and phylogeny. Bot. Rev. **8:** 247–270; 473–532; 655–707. 1942.

———. The ecotype concept. A consideration with appreciation and criticism, especially of recent trends. New Phyt. **45:** 34–43. 1946.

UITTIEN, H. Reflections on the nomenclature of so-called hybrids. Chron. Bot. **5:** 212–214. 1939.

VALENTINE, D. H. Studies in British primulas. I. Hybridization between primrose and oxlip (*Primula vulgaris* Huds, and *P. elatior* Schreb.) New Phyt. **46:** 229–253. 1947.

———. Studies in British primulas. II. Ecology and taxonomy of primrose and oxlip (*Primula vulgaris* Huds. and *P. elatior* Schreb.) New Phyt. **47:** 111–130. 1948.

VIOSCA, P. JR. The irises of southeastern Louisiana. A taxonomic and ecological interpretation. Bull. Am. Iris Soc., April, p. 3–56. 1935.

WEBER, W. A. A taxonomic and cytological study of the genus *Wyethia*, family Compositae, with notes on the related genus *Balsamorhiza.* Am. Mid. Nat. **35:** 400–542. 1946.

WETMORE, R. H. and A. L. DELISLE. Studies in the genetics and cytology of two species in the genus *Aster* and their polymorphy in nature. Am. Jour. Bot. **26:** 1–12. 1939.

WIEGAND, K. M. A taxonomist's experience with hybrids in the wild. Science **81:** 161–166. 1935.

WINGE, Ø. Artkrydsningproblemer i Planteriget. Beretning om Nord. Jordbrugsforsk. Foren. tredie Kongres, Oslo, pp. 592–606. 1926. [Not seen.]

———. The genetic aspect of the species problem. Proc. Linn. Soc. London, 150th sess., pp. 231–238. 1938.

WOLF, C. B. California plants notes. *X. Quercus morehus* Kellogg. Rancho Santa Ana Bot. Gard., Occas. Pap. **1:** 47–52. 1938.

———. The Gander oak, a new hybrid oak from San Diego County, California. Proc. Calif. Acad. Sci. **25:** 177–188. 1944.

WOODSON, R. E., JR. Notes on some North American asclepiads. Ann. Mo. Bot. Gard. **31:** 363–370. 1944.

———. Some dynamics of leaf variation in *Asclepias tuberosa.* Ann. Mo. Bot. Gard. **34:** 353–432. 1947.

ZIRKLE, C. The beginnings of plant hybridization. Morris Arb. Mono. **1:** 1–231. 1935.

Recent papers of interest

BAKER, H. G. Stages of invasion and replacement demonstrated by species of *Melandrium.* Jour. Ecol. **36:** 96–119. 1948.

BALDWIN, J. T. JR. Review of Anderson's "Introgressive Hybridization". Jour. Hered. **40:** 256–257. 1949.

CAMP, W. H. Review of Anderson's "Introgressive Hybridization". Bull Torrey Bot. Club **76:** 369–372. 1949.

ELLIOTT, F. C. *Bromus inermis* and *B. pumpellianus* in North America. Evolution **3:** 142–149. 1949.

HARRISON, J. H. Field studies in *Orchis* L. I. The structure of Dactylorchid populations on certain islands in the Inner and Outer Hebrides. Trans. & Proc. Bot. Soc. Edinburgh **35:** 26–66. 1949.

HOVANITZ, W. Increased variability in populations following natural hybridization. *In* G. L. Jepson *et al*, Genetics, Paleontology and Evolution. pp. 339–355. 1949.

MASON, H. L. Evidence for the genetic submergence of *Pinus remorata. In* G. L. Jepsen *et al*, Genetics, Paleontology and Evolution. pp. 356–362. 1949.

RICKETT, H. W. An editor's point of view. *In* Symposium on Botanical Nomenclature. Am. Jour. Bot. **36:** 10–13. 1949.

3 JAN 1969 16 OCT 1969

29 JAN 1972

INDIAN AGRICULTURAL RESEARCH
INSTITUTE LIBRARY, NEW DELHI

23 MAR 1971 23.3.74

1971 4 DEC 1972 17 JAN 1970

19 OCT 19 Date of Issue 27 NOV 19 Date of Issue 12 MAR 1970 Date of Issue